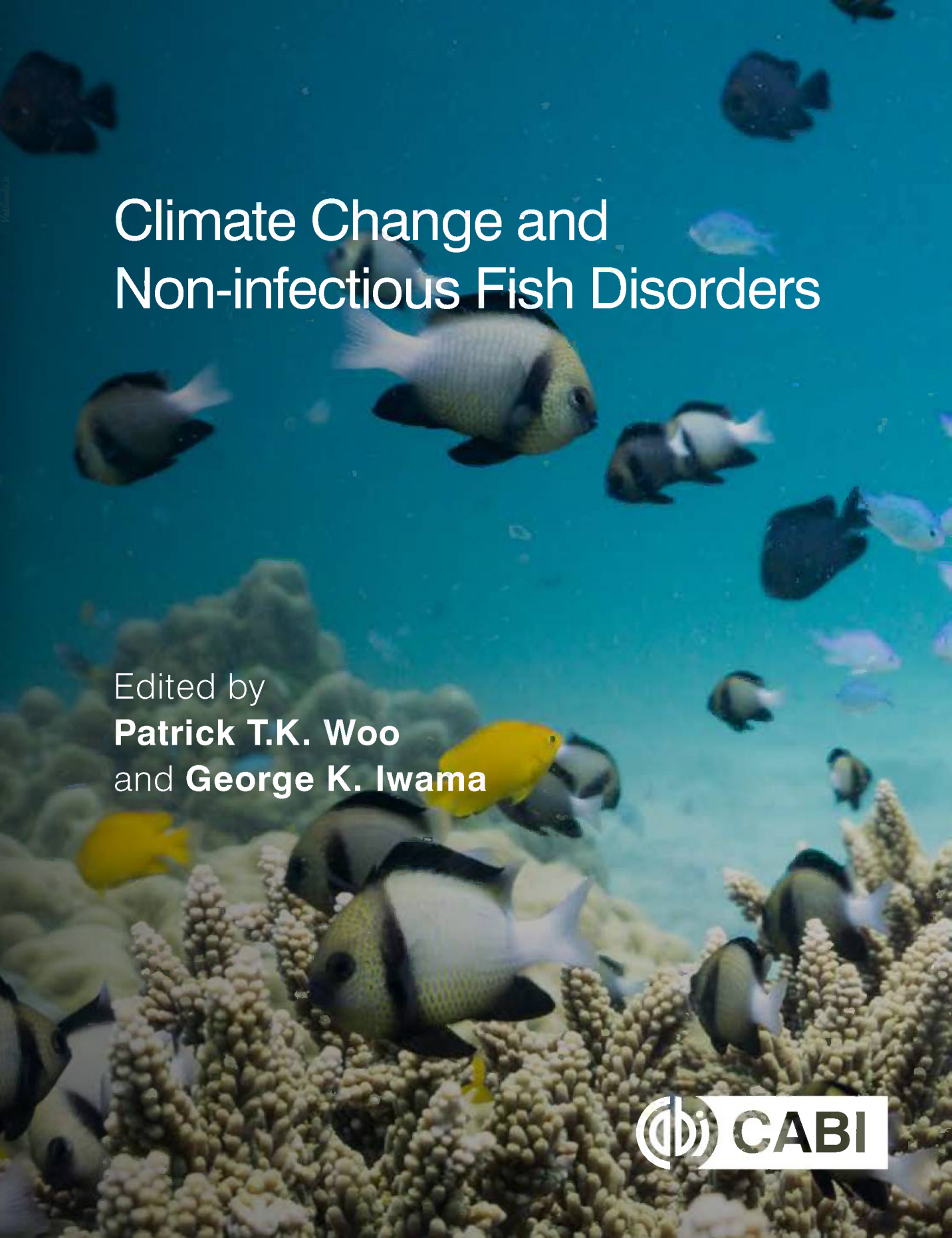


The background of the cover is a vibrant underwater photograph. It shows a variety of fish, including several black and white striped damselfish, a few bright yellow tangs, and some smaller blue fish. They are swimming over a complex, light-colored coral reef structure. The water is a clear, deep blue.

Climate Change and Non-infectious Fish Disorders

Edited by
Patrick T.K. Woo
and **George K. Iwama**



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Preface

Credible evidence on climate change with global warming is rapidly accumulating, and interpretations of available data are not disputed by reputable scientists. The aquatic environment (e.g. rivers, lakes, oceans) is greatly affected, and major contributors to climate change are carbon dioxide (CO_2 ; e.g. from the extensive use of fossil fuels in transportation, by industries) and methane (CH_4 ; e.g. from the gas and oil industry, from agricultural activities including large-scale breeding and raising of livestock for food) released into the atmosphere. These 'greenhouse gases' trap heat radiating from the Earth and thus raise the environmental temperature. Also, dissolved atmospheric CO_2 in the water not only acidifies aquatic ecosystems it also decreases the amount of dissolved oxygen at higher temperatures. This may lead to hypoxic conditions for many aquatic organisms including fish. Other negative effects associated with global warming include: (i) prolonged droughts often associated with widespread forest fires in some regions; (ii) heavier than usual rainfalls with high winds in other areas; (iii) changes in wind severity and patterns; and (iv) the increase and more rapid melting of glaciers and the North and South Poles, elevating sea levels which can modify ocean currents and salinities as well as alter aquatic food webs and the composition of animal communities. Many of these changes directly affect aquatic organisms including their development, physiology, behaviour, health and migration patterns.

The United Nations '2015 Paris Agreement' on climate change was signed by nearly 200 countries, and an important part of the pledge was to reduce the output of greenhouse gases as soon as possible so that global warming would be less than 2°C above pre-industrial levels. At present, it is about 1°C and is expected to rise to 3°C or higher if we continue with our current energy policies. The recent '2018 UN Special Report' tabled by the Intergovernmental Panel on Climate Change (IPCC; prepared by 91 authors and review editors from 40 countries) indicates that the 'Paris Agreement' needs modifications. Many of the negative impacts due to global warming would be reduced at 1.5°C compared to at 2°C or higher. Limiting warming to 1.5°C is now considered possible especially if we make concerted efforts to integrate and implement most, if not all, of the IPCC recommendations. The lower temperature increase would provide us with more time: (i) to reduce the output of CO_2 (e.g. by industries and in transportation to use less-polluting fuels, by national governments to impose a global 'carbon tax'); (ii) to provide incentives for the development of 'cleaner' fuels, and for research into renewable and sustainable energy; (iii) to develop novel and practical strategies for removal and storage of atmospheric CO_2 ; and (iv) for aquatic organisms and ecosystems to adapt to changes in the environment.

The other important greenhouse gas is CH_4 but the amount of atmospheric CH_4 is significantly lower than CO_2 . However, scientists are beginning to be concerned about the gas as a CH_4 molecule traps more heat than a CO_2 molecule. Also, the amount in the atmosphere continues to rise rapidly due to various anthropogenic activities and the concentration of the gas has doubled since the year 1800. One suggestion is that it be converted into and be removed as CO_2 .

There are simple lifestyle changes we can make to reduce our individual carbon footprint. We suggest focusing on two changes because they are within our control and consequently are achievable. Collectively, these actions (also suggested by others at various times) will have significant impact on reducing emissions of the two greenhouse gases. They are to reduce unnecessary travel (by cars and/or planes) and modify our preference from an essentially red-meat diet to either a plant-based or a fish-based diet.

Animal protein is an important component in a well-balanced diet for humans. Fish is an excellent and affordable protein for about 4.2 billion people, and we expect the demand for it will continue to increase as our global population grows. However, the information we have on the effects of environmental changes on fish and on its disorders/diseases are scattered in numerous specialized journals and reports. Consequently,

we are delighted CABI, UK has commissioned the publication of a multidisciplinary two-volume set on climate change and its effects on fish. Chapter contributors and topics for review are selected by the editors, and the chapters address current and expected changes, point out gaps in our knowledge, and articulate suggestions for future studies.

The current volume, entitled *Climate Change and Non-infectious Fish Disorders* (CCNFD) is the first of the two-volume set, and it focuses on the development, physiology and health of fish. CCNFD has 11 chapters organized into two parts. Chapters 1 and 2 (Part I) are mainly for aquatic biologists including colleagues who study non-infectious fish disorders and infectious fish diseases. These two chapters also set the stage for discussions in the remaining nine chapters. Chapter 1 presents an overview (both historical and current) on climate change while Chapter 2 is focused on abiotic changes in tropical marine and brackish ecosystems. Part II (Chapters 3–11) is on non-infectious disorders such as abnormal development, neoplasms, metabolism, feeding, the immune system, migration and overall health of fish. The companion volume to CCNFD is *Climate Change and Infectious Fish Diseases* (editors: P.T.K. Woo, J.A. Leong and K. Buchmann), and will include abiotic and biotic changes to temperate and tropical freshwater ecosystems, sequestration of atmospheric CO₂, and selected infectious microbial (12 chapters) and parasitic (10 chapters) infections on fish with strategies to minimize negative effects ongoing changes to the environment have or will have on fish health and production.

The principal audience for CCNFD includes research scientists in universities, aquatic biologists and fish health consultants in private or government laboratories. It is also useful to environmentalists and ecologists who monitor changes to the aquatic system. The book may be appropriate for the training of fish health specialists, and for graduate students and senior undergraduates who conduct field studies on fish and/or monitor changes to the aquatic environment.

Patrick T.K. Woo and George K. Iwama

Previous titles by Patrick T.K. Woo

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Edited by P.T.K. Woo and R.C. Cipriano

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An Overview with Discussions on Freshwater and Marine Ecosystems in North America

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1.1 Overview on Climate Change

1.1.1 Weather and climate change

Weather, often confused with climate, are changes on an hourly, daily, weekly or monthly basis. Atmospheric temperatures may readily vary by as much 20°C within a single day. These are short-term changes with high variances. However, climate change describes large-scale changes from yearly to decadal to millions of years. It is an average of weather temperatures on an annual basis over long periods of time. Temperature change is part of what characterizes climate change. There are many aspects to climate change, of which changes in precipitation patterns, severe storm frequency and changes in average annual temperature, among others, are a part. Throughout geological history, Earth has experienced profound average temperature changes, with both intense increases and decreases, including those which precipitated the Ice Ages (McInerney and Wing, 2011; Engber, 2012). At this time in the history of Earth, we are experiencing higher temperatures over the entire planet, some more severe than others. For example, the northern hemisphere is warming faster than the southern hemisphere, particularly the Arctic and subarctic regions (Feulner *et al.*, 2013); this is termed 'global warming'.

1.1.2 Atmospheric warming and atmospheric carbon dioxide

Concentrations of atmospheric carbon dioxide (CO₂) and other 'greenhouse gases' have been increasing to levels unprecedented over the last 800,000 years (World Meteorological Organization, 2017) and are now known to be responsible for the rise in average global temperatures that we are currently experiencing. This rise began during the Industrial Revolution, became particularly prominent during the 1930s and 1940s, and has been increasing ever since. The National Aeronautics and Space Administration's (NASA) Goddard Institute reports that global average temperatures have increased by ~0.9°C since 1880 (NASA, 2018) and are forecasted to continue to increase to at least 1.5°C above pre-industrial levels (IPCC, 2014); pre-industrial defined as the time prior to 1750. After 1750 England began burning abundant quantities of coal. Pre-industrial CO₂ values have been reported at 278 ppm (Forster *et al.*, 2007) increasing to ~392 ppm in 2011 (NOAA, 2011). Levels continued to increase and in 2017 were reported as 407.72 ppm, increasing to 409.22 by April 2018 (see NOAA, 2018) representing an annual mean 0.11 ppm/year growth rate. Tillmann and Siemann (2011) suggest projected levels may reach ~650 ppm by 2100 and perhaps exceed 1000 ppm

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in time (also see Meehl *et al.*, 2007), causing catastrophic warming/heating consequences on ecosystems globally.

As atmospheric CO₂ increases the oceans absorb ~40% of it (World Ocean Review, 2010) causing decreases in the pH of oceans worldwide (Orr *et al.*, 2005; Riebesell and Gattuso, 2015). The decreased pH has a negative effect on the ability of many aquatic organisms (e.g. bivalves, corals) to effectively calcify processes, which, in turn, affects their survival. Feely *et al.* (2009) for instance suggest that pH in the oceans worldwide has already decreased from 8.2 to 8.1 (pre-industrial versus current values, respectively). Feely *et al.* (2010) also suggest that over the next 100 years, pH may decrease from 8.1 to 7.8. Should this happen, many organisms that depend upon aragonite for calcification will reach a critical threshold and be unable to form shells and for those that already have a shell, those will begin to dissolve as the water becomes more acidic. Less known are the effects of increasing atmospheric CO₂ on freshwater lakes and rivers (Hasler *et al.*, 2016). However, a recent study conducted by Weiss *et al.* (2018) over a 35-year period (1981–2015) examining two species of *Daphnia* (water fleas) in four reservoirs located in Germany showed a ~0.3 decrease in pH resulting in poor predator detection and decreased self-defence mechanisms against predation. Ou *et al.* (2015) similarly showed that decreases in pH in a freshwater habitat has negative consequences on pink salmon (*Oncorhynchus gorbuscha*), causing anti-predator behaviour and reductions in growth. The biological consequences of increased acidity in our aquatic ecosystems is impaired olfactory discrimination (Wisenden, 2000; Munday *et al.*, 2009; Dixon *et al.*, 2010; Midway *et al.*, 2017) and appears to affect many different types of organisms in both marine and freshwater habitats.

1.1.3 Milankovitch cycles, seasonal changes

Global shifts between warming and cooling on Earth have been described as Milankovitch cycles (Rahmstorf and Schellnhuber, 2006), driven by variations in the orbital and axial movements of the Earth as it revolves around the sun. According to palaeoclimatological studies, warming or cooling events occur about every 100,000 years (Keeling and Whorf, 2000; Climate Literacy, 2009; Toggweiler and Lea, 2010) and are considered to be normal. According to this model, the Earth should be in a

natural cooling cycle (OSS Foundation, 2018); however, since approximately 1850 (coinciding with the beginning of the Industrial Revolution), there has been a significant increase in the amount of CO₂ added to the atmosphere and, as a consequence, rather than cooling, Earth has been deviating from its natural cycle and following a steady warming trend (OSS Foundation, 2018).

Over the last century, average atmospheric temperatures in western North America have increased by ~0.6–1.0°C (Tillmann and Siemann, 2011). In Alaska, temperatures increased by as much as ~1.9°C between 1949 and 2009 (US Global Change Research Program, 2009). Many investigators have projected that by 2100, the Earth will experience severe heat increases (see IPCC, 2013). For example, Peterson and Schwing (2008), Mote *et al.* (2010) and PRBO Conservation Science (2011) suggest temperature changes ranging from 1.5°C to as high as 7.2°C, with the greatest increases in the most northerly latitudes (e.g. Alaska). A compendium written by numerous researchers (IPCC; Intergovernmental Panel on Climate Change) indicate that while many people (including scientists, politicians, etc.) dismiss these predictions of changes as being alarmist, even the most conservative mathematical models of climate change predict that some increase in temperature change is likely. While most people hope for the least amount of change (i.e. 1.5°C), modelling suggests temperatures in excess of 6°C are more likely.

At the surface of this issue, many scientists are concerned about the potential increase in frequency and duration of each warming event (Union of Concerned Scientists, 2018a). What is more disconcerting is that, according to geological predictions, we are supposed to be in a cooling trend; but instead, the planet is warming (Budyko, 1972; Herring and Simmon, 2007; Phipps cited in Andrews, 2016). This suggests that the next natural cycle will be a warming trend, thus compounding the problem. Gerald Meehl (National Center for Atmospheric Research) suggests that over the last few decades, the number of warming trends is approximately twice the number of cooling trends across the USA, but by 2050, that ratio is expected to increase from 2:1 to more than 20:1 (warming versus cooling, respectively) (Meehl *et al.*, 2007). The number of warming versus cooling events from weather stations scattered across the contiguous USA show more warming events since the 1980s compared with cooling trends (Fig. 1.1).

As the number of warming days outpaces the number of cooling days, seasons will begin to shift (Fig. 1.2) with earlier springs, longer summers and autumn weather beginning later in the year. In fact, in the northern hemisphere, over much of the continental USA, winter is becoming shorter in duration due to warming weather conditions with temperatures in many northern states, such as Montana, Minnesota and Michigan which are experiencing temperatures well above normal mean maximums characteristic of the 1970s. In some of the

more southerly states such as California and Texas, seasonal shifts are already being observed during the spring and autumn seasons (Fig. 1.3).

It is easy to confuse wide variations in weather conditions and climate change. By following average temperatures over long periods of time, it is clear the Earth is becoming warmer. As the atmosphere warms, positive feedback loops are induced, and warmer air will produce increased precipitation (Fig. 1.4).

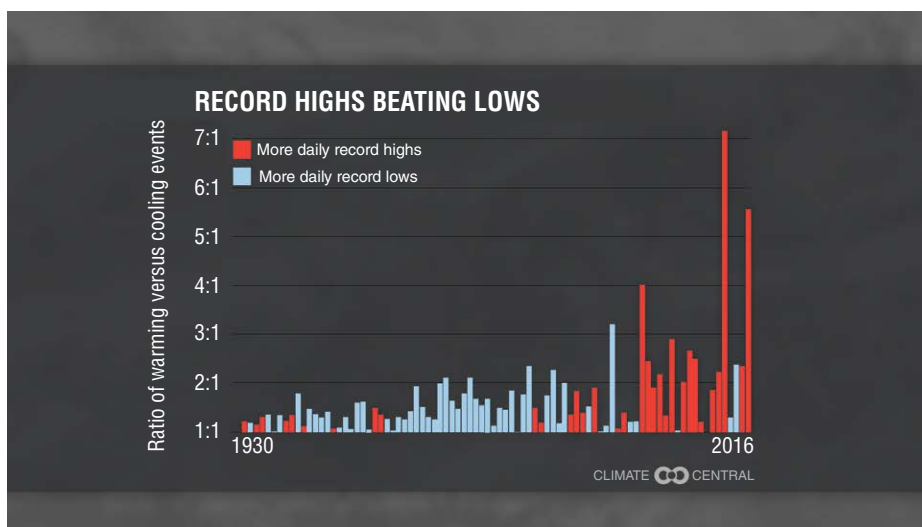


Fig. 1.1. The number of high warming trends outpacing the number of cooling trends from 1930 to 2016. (Reprinted from Climate Central, 2017, with permission.)



Fig. 1.2. As warming trends begin to outpace cooling trends each decade, expected changes include shifting seasons where summer lasts longer. (Adapted from Climate Central, 2017, with permission.)

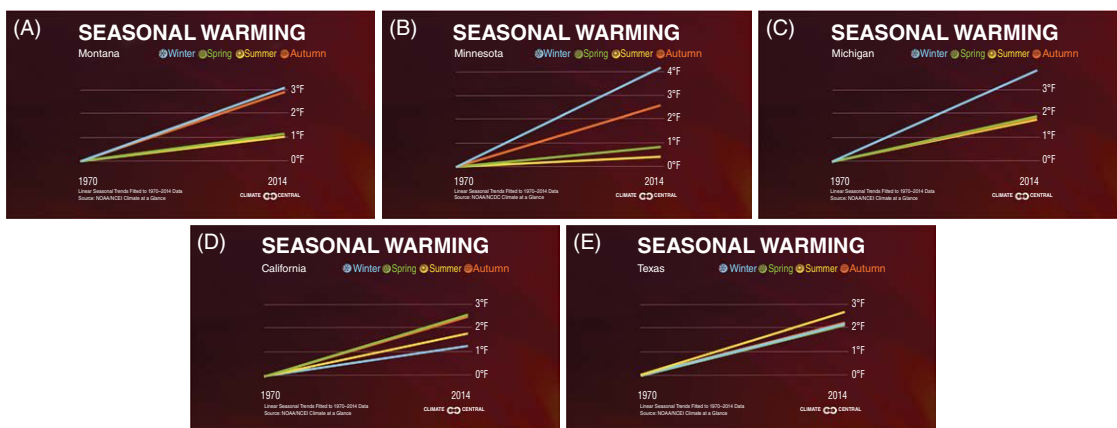


Fig. 1.3. Season mean temperatures from the 1970s through to 2014 are compared in (A) Montana, (B) Minnesota, (C) Michigan, (D) California and (E) Texas. (Adapted from Climate Central, 2017, with permission.)

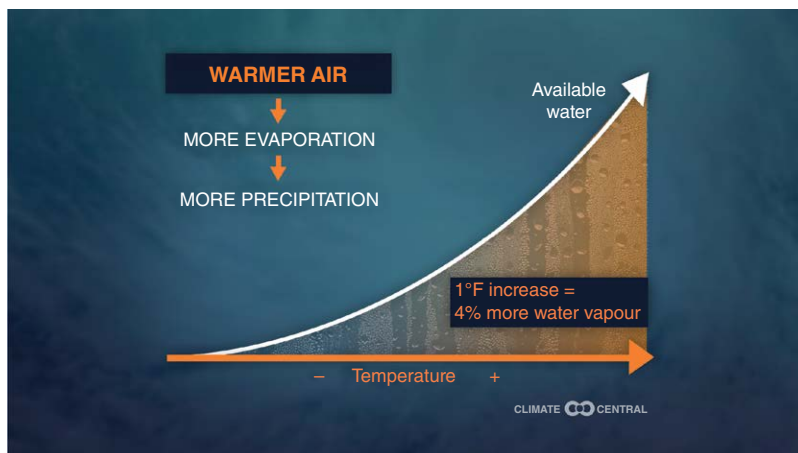


Fig. 1.4. The relationship between the warming atmosphere and its effect on precipitation. (Adapted from Climate Central, 2017, with permission.)

If the Earth's climate continues to warm, this will affect both our terrestrial and marine climatic zones, their environmental characteristics (e.g. polar ice caps), and the organisms that reside within them. For instance, for every 0.6°C (1.0°F) increase in temperature, the atmosphere can hold 4% more water vapour (Climate Central 2017). As a consequence, rather than equal volumes of snow being observed in northern climates/latitudes from year to year, more rain (rather than snow) may be experienced. In more tropical areas, that does not necessarily mean there will be more precipitation, mainly because the atmosphere in those areas can

hold that water without condensing and producing rain. This is why the Sahara Desert has been expanding in recent decades (Thomas and Nigam, 2018; University of Maryland, 2018).

In the northern latitudes, the polar ice caps, which are the result of snow accumulated over thousands of years, will also decrease as a result of this warming. As snow and ice continue to melt and disappear, the reflectivity or albedo (Zeng and Yoon, 2009) of the Earth will decrease and more radiant energy from the sun will be absorbed by the land and the waters. The northern hemisphere possesses more land than the southern hemisphere,

and the question arises whether there will be less heating in the former. Land more readily absorbs heat than water; hence, the northern hemisphere is expected to absorb more heat. Consequently, the northern hemisphere is expected to warm at a higher rate than the southern hemisphere, and this is what is currently being observed (Cook, 2018; NOAA, 2018).

1.1.4 Latitudes of change

Receiving different amounts of sunlight, latitudes are actually angular measurements that begin at the equator with a value of 0° , extending to the poles at 90° (The Environmental Literacy Council, 2015a). Depending upon a region's latitude and proximity to the sun, the climate conditions will vary depending upon the angle of the sun's rays. Hence, higher latitudes receive less heat from the sun compared with lower latitudes because the angle at which the sun strikes the Earth is higher than the angle experienced near the equator. Because the Earth is tilted 23.5° to the perpendicular, the seasons shift from hemisphere to hemisphere as the Earth revolves around the sun. For example, the northern hemisphere is tilted towards the sun from March to September and consequently receives more solar energy and heat than the southern hemisphere. In terms of climate change and global warming, and despite receiving less heat due to proximity to the sun, Freedman (2013) suggests that warming in the northern hemisphere is occurring much faster than in the south not only because of increased greenhouse gas emissions but additional warming due to global ocean currents 'pulling' and transporting warmer waters from the south and transporting them to the north (also see Levke *et al.*, 2018). In the coming decades, Friedman *et al.* (2013; see Fig. 1.5)

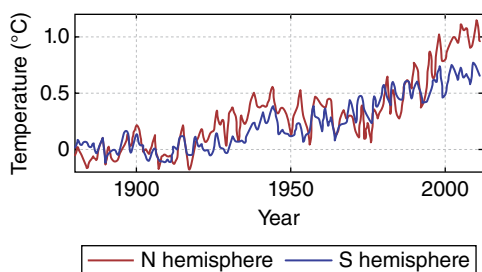


Fig. 1.5. Warming trends comparing northern (N) versus southern (S) hemispheres. (Adapted from Friedman *et al.*, 2013.)

suggests that the north will continue to warm faster than the south, and the differences in temperature will be much more noticeable.

Tropic zones have been described as falling between 23.5°S and 23.5°N . These are the limits between which the sun's rays impact the Earth at a 90° angle, moving between these latitudes with every revolution around the sun. While this zone receives the most sunlight, it may not necessarily be the warmest region, due to the larger ocean mass in the southern hemisphere compared with the northern hemisphere, and the comparative abilities of these regions to absorb solar energy. Between the Tropic of Cancer (23.26°N) and the Arctic Circle (66.34°N) lies a major climatic zone – the north temperate zone. This is mirrored in the southern hemisphere by the Tropic of Capricorn ($\sim 23.26^\circ\text{S}$) and the Antarctic Circle ($\sim 66.34^\circ\text{S}$). Collectively, the temperate zones are populated by the majority of the population (Fig. 1.6; Rankin, 2008).

Mascarelli (2013) suggested that climates will indeed change on the Earth; he predicted that the number of warmer climates on the Earth will nearly double by the end of the century, and that approximately one-fifth of the Earth's land mass will experience some degree of climate shift. He also states that while the polar zones retract over time, other regions will experience fewer cool summers. Consequently, organisms will move or disperse (via reproductive propagules) and relocate to new habitats with environmental characteristics that fall within their physiological tolerances. Endemic species within a stressed region will acclimatize, adapt or become extinct.

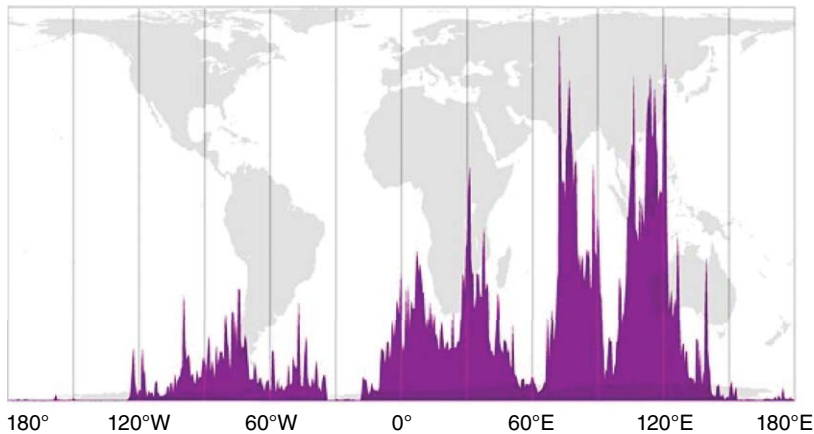
Will polar movements of some of the terrestrial climatic zones be accompanied by shifts in the jet streams? There are four major jet streams with two oscillating in their movements around the 30°N and 30°S latitudes (polar jets). The other two are subtropical jets that oscillate around the $50\text{--}60^\circ\text{N}$ and S latitudes (Fig. 1.7; OSS Foundation, 2018). Climate has a significant effect on these jet streams, which in turn, affect weather. Although these jet streams may oscillate differentially over the course of days, months and years, their meanderings are relatively stable within certain latitudinal bounds (OSS Foundation, 2018). It has been reported that the jet streams are slowly changing, and shifting as predicted (OSS Foundation, 2018) – moving poleward (Fig. 1.8; Climate Central, 2013). The impact to all organisms and habitats is that as the jet streams shift, so do the regional weather patterns (Hudson, 2012).

The World's Population in 2000, by Latitude



(horizontal axis shows the sum of all population at each degree of latitude)

The World's Population in 2000, by Longitude



(vertical axis shows the sum of all population at each degree of longitude)

Fig. 1.6. World population by latitude versus longitude. (Reprinted from Rankin, 2008, with permission.)

Aspects of both observed and predicted climate change have been considered in detail for terrestrial ecosystems, and many studies have compared future developments for these habitats (e.g. Franklin *et al.*, 2016; Hölzel *et al.*, 2016). We will consider the potential effects of climate change in marine and freshwater habitats in the following sections.

1.1.5 Temperate climate change, water resources and fisheries

Water, essential to all of life covers approximately two-thirds of the Earth's surface (The Environmental

Literacy Council, 2015b). Approximately 97% of that water is salt water and 3% is fresh water, but only 1% of the fresh water is readily available while the remainder is either locked up deep underground or in glaciers and ice caps. Despite this scarcity of fresh water, 40% of the world's fish species are found in freshwater habitats (Tedesco *et al.*, 2017) where temperatures vary from 2°C in the winter to summer temperatures as high as 24°C (Santhosh, 2018). Limited information, however, is currently available describing how these freshwater ecosystems will be affected by climate change. Most climate change models examine the effects of global

warming on the oceans, despite the existence of more than 100 million lakes. The IPCC has suggested that freshwater habitats are among the most vulnerable to climate change.

1.2 Freshwater Ecosystems

Global warming is already affecting freshwater aquatic habitats. These include: (i) warming of aquatic surface waters of lakes and streams (Poff *et al.*, 2002); (ii) reductions of ice cover associated with lakes and rivers (Hewitt *et al.*, 2018); (iii) melting of glaciers and permafrost (Beniston *et al.*, 2018); (iv) increases in the hypolimnetic temperatures of deep lakes and rivers (Hershkovitz *et al.*, 2013); and (v) changes in the freshwater biota (Döll *et al.*, 2018), particularly fish (Ruby and Ahilan, 2018). For

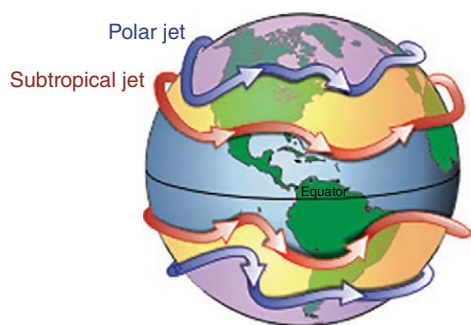


Fig. 1.7. Subtropical and polar jet streams. (Reprinted from OSS Foundation, 2018, with permission.)

instance, it has been reported that coldwater fish are decreasing in the Great Lakes, due to less ice coverage than in prior years, increased algal blooms, and a greater expanse of hypoxic regions in the lakes (Mysak, 2016).

In 2015, O'Reilly *et al.* (2015) studied more than 235 lakes worldwide and reported average lake water temperatures increased by approximately 0.34°C (0.61°F) every 10 years between 1985 and 2009. While some lakes gained heat energy, others apparently cooled. No clear geographical boundaries could be identified, because the effects (warming versus cooling) were scattered among latitudes. Also, depth and size of the lakes contributed to much of the variation. For instance, Lake Superior (part of the Laurentian Great Lakes Basin) increased in temperature by 1.0°C per decade. The other Great Lakes (e.g. Lake Erie), while also showing a warming trend, were slower in their responses (e.g. 0.1°C over the same period; O'Reilly *et al.*, 2015). In the case of Lake Superior, scientists believe the reason for a more pronounced warming is that it is becoming more stratified earlier each year. According to Witze (2017) Lake Superior previously stratified in mid- to late July, but now this is occurring in June. Other lakes like Lake Erie are warming at a much slower pace, in part because it is shallower (64 m) in comparison to Lake Superior (406 m) and stratification is less affected because heat is transferred all the way to the bottom and thus stratification will not occur. In Canada, MacDougall *et al.* (2018) studied 721 lakes at an 11° latitudinal gradient and

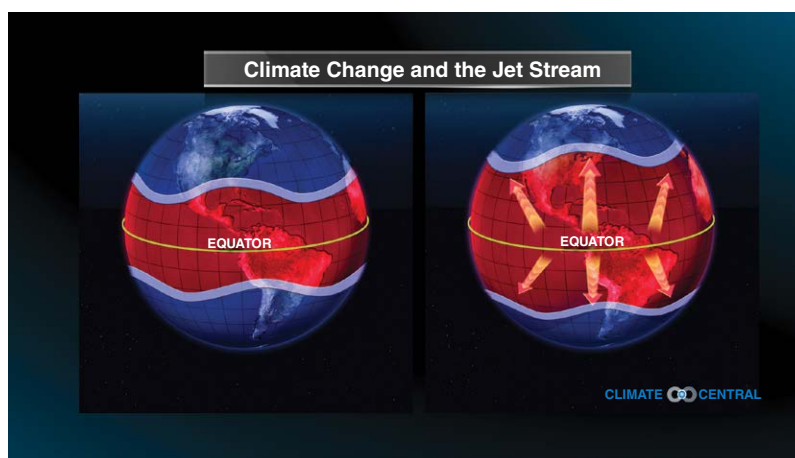


Fig. 1.8. Expected poleward movement of the jet streams in response to warming of the atmosphere as part of climate change. (Reprinted from Climate Central, 2013, with permission.)

described that while predator–prey interactions are important, the environment (i.e. climate change) had a much greater impact on species richness. While changes in temperature (i.e. 0.1–0.2°C) may appear to be small, researchers of many lakes around the world demonstrate what some of the negative impacts of these changes can be in the long term. Witze (2017) described changes in a lake in east Africa (Lake Tanganyika) where an increase of 0.2°C per decade has resulted in less mixing, more stagnation, and nutrients becoming trapped at the lake bottom near the benthic community, resulting in biological productivity decreasing substantially (e.g. sardine catches have dropped by 50%). Lake Poopó (Bolivia), Lakes Chad and Tanganyika (Africa), and Lake Urmia (Iran) are examples where temperatures have increased, lake volumes have decreased, and the plant and animal species are disappearing (Weiss *et al.*, 2018). Read (limnologist, US Geological Survey, Middleton, Wisconsin; cited in Witze, 2017) and colleagues examined more than 2000 lakes in Wisconsin (USA) from 1989 to 2014 and describe how fish populations have changed over this period. Many of these lakes experienced decreases in walleye (*Sander vitreus*) fish populations. Projecting how lake temperatures change through to 2089, these authors describe how major decreases in walleye populations would most likely occur in more than 75% of the state's lakes. These results have been supported by Hansen *et al.* (2016) who similarly predict a natural decline of walleye, which prefer cooler waters, versus largemouth bass (*Micropterus salmoides*), which prefer warmer water. Hansen *et al.* (2016) also concluded that lake temperatures will increase and further suggest that if greenhouse gas emissions continue to escalate, temperatures may exceed 2.8°C (5.0°F) by 2090. With lower greenhouse gas emissions, they project that lake temperature may only reach ~2.5°C above mean current averages by the year 2090.

In the Great Lakes, ice cover has steadily declined since the 1970s (Climate Central, 2016). This lowered ice cover results in warmer surface waters, which then compounds problems of pollutants, algal blooms and the quality of drinking water. The vast volumes of water (oceans and, to a lesser degree, freshwater lakes, etc.) absorb and retain a great deal of heat energy from the sun. On an annual basis, the upper ocean layers are considered to have absorbed more than 43 times (i.e. 43 ×) the total amount of energy consumed by the US population in 2012 (last year data was available).

Notaro *et al.* (2015) claim that in the short term, the Great Lakes region will accumulate more lake-effect snow; they will also, however, remain ice free longer in the autumn and winter with earlier ice break-up in the spring. As this region continues to warm and experience greater evaporation, the long-term precipitation trend will be for less snowfall, more rainfall, and delayed or shortened frost periods. In the Arctic, should an ice-free summer occur, many authors have indicated this would cause a collapse in plankton blooms which serve as food for birds, fish and whales (Berwyn, 2017). Examining the magnitude and speed at which climate change is occurring, Comte and Olden (2017) compared 80 years of marine versus freshwater laboratory experimental data involving ~2960 ray-finned fishes (~485 species) to thermal sensitivity. They suggested the data showed significant thermal sensitivity to tropical marine fishes and freshwater fishes located at higher latitudes in the northern hemisphere; these fishes will either relocate, rapidly adapt or acclimatize, or the local population(s) will die. Considering the frequency and duration of each warming trend, and the evolutionary trends regarding how quickly a species can adapt, we predict that many fish species will be unable to cope with such changes. As climate change and global warming continues, many scientists predict: (i) increases in stream and river flow (based on changes in seasonal intensity and distribution of rainfall) (Yin *et al.*, 2017; Worqlul *et al.*, 2018); (ii) increased precipitation, flooding and evaporation (The Climate Reality Project, 2017; Wang *et al.*, 2017); (iii) as evaporation continues to become exacerbated, the disappearance of some lakes and streams (United Nations Environment Programme, 2018); and (iv) increased frequency of pathogen outbreaks (Taylor *et al.*, 2018; Zhan *et al.*, 2018) and algal blooms, and decreases in the abundance and diversity of particular species.

While in some ways these changes might be welcome to some, for example farmers across the USA have experienced growing seasons longer than usual by nearly 2 weeks and earlier springs (Kunkel, 2016), parallel changes are not welcome to others. In colder climates, long winters support local economies via ice fishing, hockey, snowmobiling and other outdoor activities. In addition, the 'deeper freeze' helps control disease. Milder winters will allow increased survival of disease-carrying pathogens (e.g. Lyme disease – Pfeiffer, 2018; mosquitoes – European Commission Joint Research Centre, 2018) and increased respiratory illnesses caused from allergies (Staudt *et al.*, 2010). Many fruit-bearing

and other trees will bloom earlier, and it is not known whether pollinators will be able to adapt to these temporal changes. The fruit-bearing trees and plants may have smaller yields because of this.

In colder climates, like the northern latitudes, primary productivity is expected to increase because of reduced ice coverage, greater absorption of heat, and increased nutrient loading (European Environment Agency, 2012). It is also likely that there will be population increases in particular organisms, especially invasive ones, and a decrease in endemic species. Because of these changes, Jappesen *et al.* (2009) predicts that significant changes will occur in food web structures, and quite likely changes in dissolved oxygen content. The European Environment Agency (2012) suggests that it is unlikely that our attempts to remediate these changes and restore lakes and estuaries to their prior state will succeed. They suggest that such attempts will most likely be confounded by an ecosystem already responding to environmental changes via adaptation. As lakes begin to increase in temperature and duration of warmer periods, it is expected that oxygen depletion in the hypolimnion will occur as populations of algal species increase and create a negative feedback loop where increased nutrients are added to the water through their death, leading to hypoxia in the benthos.

As mentioned earlier, food webs will also probably be affected with the warming of our waters. Reduced ice cover, for instance, will likely enhance population growth in some fish species and cause the extinction of others. It should be no surprise that as northern temperate waters warm up, cold-water fish will be replaced by fish better adapted to live, grow and reproduce at those temperatures. Some fish and other species living in these regions will need to migrate northwards, and they will most likely be replaced by other species moving northwards from further south. Some cold stenothermic invertebrates and many salmonid species will suffer losses in this way and are expected to decrease in both population abundance and species diversity (European Environment Agency, 2012).

The European Environment Agency (2012) predicts water temperatures to increase in lakes and rivers with a shift in food web dynamics. They predict that there will be earlier spring blooms of phytoplankton and zooplankton, and a switch from a more dominant zooplankton and macrophyte type of ecosystems to dominance by bloom-forming phytoplankton and those fish species that

can take advantage of such a food resource. Increased precipitation will also lead to increased runoff, which will increase nutrient loading and trigger more algal blooms. These changes may or may not serve as an acceptable food source for fish and other freshwater organisms. Natural mortality in these algal populations and the ensuing bacterial breakdown will deplete the oxygen resources in affected areas. The Union of Concerned Scientists (2018b) suggests that, as this occurs, we can expect the formation of larger hypoxic (dead) zones. In a freshwater habitat like Lake Superior, scientists believe this vast body of water is beginning to show the effects of progressive warming caused by rising water temperatures and increased evaporation levels (Witze, 2017).

Overall, global climate change and warming temperatures are likely to result in positive feedback loops with physical, chemical and biological changes through space and time. Such changes will not be simple or linear but rather, complex – resulting in ‘winners, losers, and surprises’ (Fulton, 2011).

1.3 Marine Ecosystems

1.3.1 Physical limitations for tropical corals, and how they will be affected by the temperate climate

It has been suggested that corals in tropical and subtropical climates will be able to colonize those oceanic regions currently classified as temperate or sub-temperate, as the latter regions are encroached upon by the former (Sammarco and Strychar, 2016). The tropical regions are expected to expand to the north and south, displacing the temperate and sub-temperate oceanic climatic regions. Indeed, there appears to be some evidence that this is already occurring. Some coral reefs have been observed developing off Broward County, Florida (Vargas-Angel *et al.*, 2003; Precht and Aronson, 2004). This is north of Key Biscayne in the USA, which was previously believed to be the northern limit of coral reefs in this region. In addition, coral reefs have now been observed in the northern sections of the Ryukyu Islands of Japan, where they had not been seen before (Yamano *et al.*, 2011). These researchers estimate the rate of expansion in this area to be up to 14 km/year. This may need to be verified, but the fact remains that several examples of a poleward expansion of coral reefs under current global warming conditions exist.

The expansion of coral reefs into previously temperate or sub-temperate regions is expected to have a latitudinal limit. To what extent this poleward encroachment will proceed remains to be seen. The rate at which it occurs will be limited by reproductive success, larval dispersal and larval recruitment – and this will be at least partially environmentally mediated.

1.3.2 Light

That limit of northward and southerly expansion of coral reefs into previously temperate regions will most likely be limited by three physical factors. The first involves the amount of light penetrating to the benthic surface. As one moves further north from the Tropic of Cancer and south from the Tropic of Capricorn, the sun's rays impact the sea at an angle which decreases with increasing distance from the equator (Harris, 2018). The amount of light reaching a zooxanthellate coral is critical to its survival. The second factor is the character of the wave spectrum associated with that decreasing light. As that angle decreases, light passes through more and more water to penetrate to a given depth. As the maximum depth of light penetration decreases, the colour spectrum reaching the benthic surface changes, becoming

increasingly blue which is similar to the blue water in deeper mesophotic zones (University of Hawai'i, 2018; Fig. 1.9). This light spectrum is not well suited to the growth of shallow-water tropical corals. The third physical factor which will most likely limit the shifts in temperate and tropical climatic zones is a seasonal one. As the seasons move from summer to winter, day length grows increasingly shorter in the winter. Shorter day lengths translate to less light per day for any benthic algal-symbiotic organism, such as a coral. Thus, not only will the light penetration become more limited, and the light spectrum be changed with latitude, so will the total amount of light available to shallow-water or deep-water corals during the late autumn, winter and early spring periods (Fig. 1.10; Fondriest Environmental Inc., 2014).

1.3.3 Temperature

Another limiting factor will be seawater temperature. Corals thrive within a narrow temperature range – ~18–28°C (Levinton, 2017). Much recent research regarding temperature tolerance has focused on temperatures increasing beyond the known limits for corals. This has been spurred by the devastating mass coral bleachings observed to

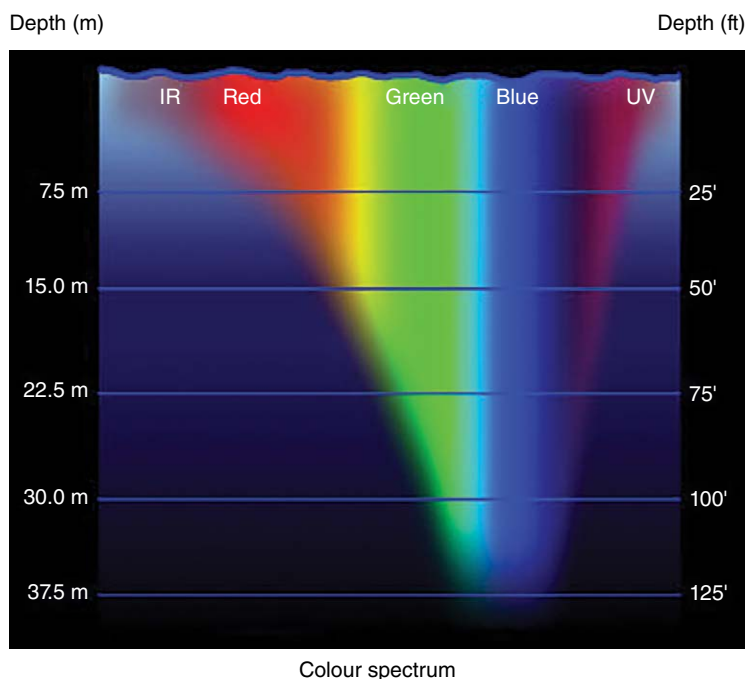


Fig. 1.9. Illustration of how the colour spectrum of sunlight changes with depth in the ocean. As the sun's angle impacting the Earth becomes more acute, the amount of the light reaching a given depth diminishes, and the light spectrum becomes more blue. IR, infrared; UV, ultraviolet. (Modified from Scuba-Monkey.com (<https://www.scuba-monkey.com/wp-content/uploads/2013/08/light-absorption-by-water.jpg>).)

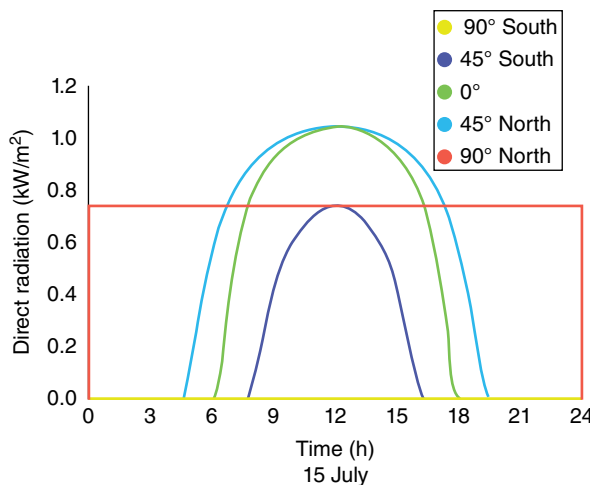


Fig. 1.10. Differences in direct solar radiation (measured by irradiance) during the summer (15 July) as a function of time over a 24 h period. Note that radiation is highest over the equator and the hemisphere tilted towards the sun. (Used with permission, <https://www.fondriest.com/environmental-measurements/parameters/weather/photosynthetically-active-radiation/>.)

occur repeatedly since the early 1980s (Goreau and Hayes, 1994; Hoegh-Guldberg *et al.*, 2007; Hughes *et al.*, 2017). Much less attention has been paid to the lower limit of the corals' temperature range (Coles and Jokiel, 1978; Coles and Fadlallah, 1991; Saxby *et al.*, 2003). Yet this factor will most likely help us to understand what may define the latitudinal limit of expansion of tropical and subtropical corals into the current temperature zones. In the Abrolhos Islands, Australia, zooxanthellate corals can exist below 18°C, but their growth rates are diminished (Johannes *et al.*, 1983). Their calcification rates, however, remain stable at these marginal cool temperatures (18°C). Competition for space with benthic algae in these sub-temperate waters was determined to be the limiting factor for further southerly expansion of the coral community. These factors may well contribute to setting the limit for the southerly and northerly expansion of corals under current climate change/global warming conditions.

This point is best illustrated by an example from the National Oceanic and Atmospheric Administration (NOAA) Flower Garden Banks National Marine Sanctuary, Gulf of Mexico where there are the two most northerly coral reefs. They occur at the edge of the continental shelf, approximately 185 km south-west of Galveston, Texas, USA. They possess well-developed coral communities and are generally protected from coral bleaching, which has decimated other reefs in the Caribbean and around the world. This protection is generally attributed to their distance from shore, associated insulation from contaminants derived from groundwater runoff, and from the warm summer seawater temperatures

associated with shallower depths which may surpass their temperature tolerances (e.g. 31.1°C, eastern Gulf of Mexico, July–August 2017; NOAA, 2018). The development of these two coral reefs may well be attributed to the Yucatan (Gyory *et al.*, 2013) and Loop Current (Texas Pelagics, 2016) of the Gulf of Mexico, both of which are derived from the Caribbean Current. These currents impact the edge of the continental shelf and bathe these banks in warm Caribbean seawater year-round. Because of this, seawater temperatures remain within the physiological tolerance limits of corals and other zooxanthellate organisms, providing them with an environment conducive to calcium carbonate reef development.

On the other hand, the Stetson Banks, which are only 48 km to the north-west of the Flower Garden banks, and ~157 km from shore are not true coral reefs. They are comprised of uplifted layers of claystone and sandstone (Lankford and Curray, 1957). The cooler winter temperatures, which fall below 18°C there, do not permit the tropical and subtropical corals to contribute substantially to the development of a calcium carbonate cap. Thus, only a few degrees difference in temperature, at the lower end of the corals' temperature range, can limit or thwart reef development.

1.3.4 Replacement of zooxanthellate corals by azooxanthellate corals in the reef community?

Could temperate ahermatypic/azooxanthellate corals replace zooxanthellate corals, colonizing warmer

habitats in lower latitudes? Azooxanthellate scleractinian corals exist throughout the world's oceans (Freiwald, 2002). They can disperse successfully, as do the zooxanthellate corals, and this has been demonstrated through their dispersal to and colonization of offshore oil and gas production platforms in the Gulf of Mexico (Sammarco *et al.*, 2012a, b). The only azooxanthellate species which have been able to proliferate to an extraordinary degree and dominate some benthic communities in the Gulf of Mexico are the invasive Indo-Pacific corals *Tubastraea coccinea* and *Tubastraea micranthus* (Sammarco *et al.*, 2010, 2012a, b, 2014). These species have been able to monopolize benthic substratum within several years of colonization. Ahermatypic corals, particularly those in deep water and in temperate and polar climatic zones also possess colder temperature tolerances than their hermatypic counterparts. In summary, it would appear that ahermatypic corals will remain an important part of the benthic community as global warming continues to increase. They will not, however, assume the same niche as the hermatypic scleractinian corals, since they will not be significant contributors of calcium carbonate to the benthic substratum. In general, their growth will be less extensive than those of their hermatypic counterparts. Invasive azooxanthellate coral species such as *T. coccinea* and *T. micranthus* will retain their ability to dominate the benthos in isolated habitats. They will not, however, build calcium carbonate-based reefs, as their hermatypic counterparts, and if the oceans waters warm to 34°C, they will not survive (Strychar *et al.*, 2005).

1.3.5 Mesophotic reefs as potential larval sources for shallow-water reefs

It has been suggested that those zooxanthellate corals which die in shallow water due to rising seawater temperatures could be replaced by zooxanthellate corals which live in deeper, somewhat cooler and darker waters on mesophotic reefs (Serrano, 2013; Laverick *et al.*, 2016). These reefs are insulated by depth from high-temperature environmental perturbations. Mesophotic reefs may be defined as follows: 'Mesophotic coral reef ecosystems (MCEs) occur in tropical regions extending from depths of 30 m to the limit of zooxanthellate corals (approx. 150 m)' (Ocean Research and Conservation Group, 2018). They are characterized by light-dependent coral, algae and other organisms that are found in

water with low light penetration (Sammarco *et al.*, 2016). It has been suggested that mesophotic reef communities may be connected to shallow coral reef ecosystems, and that they may provide an important source of larvae for threatened coral and fish populations in shallower water, and, indeed, there is some genetic evidence for this (van Oppen *et al.*, 2011).

This concept may be correct, to a certain degree; but there are several constraints regarding recolonization from this deeper-water coral community in the event of mass shallow-water mortality. First, corals which exist at these deeper depths are adapted to grow and reproduce in that environment. The endosymbiotic zooxanthellae of shade-adapted corals, or deep-water corals, have more chlorophyll per zooxanthellar cell, being adapted for very efficient photon capture in a region where light is scarce (Porter *et al.*, 1984). There is evidence that these populations would adapt to new environmental conditions (Dustan, 1979, 1982). Secondly, the species diversity of corals is lower in deeper waters and they represent a smaller proportion of the shallow-water coral community (Bak *et al.*, 2005; Kahng *et al.*, 2010; Bongaerts *et al.*, 2017). There are some species overlaps between the deeper and shallower habitats with some species which only occur in these environments (Loya, 1972). It is possible that the latter may not be adapted to shallower water. Thus, the question of recolonization from deeper waters remains unknown and needs further research. Replacement of dead or dying coral communities vertically from deep-water reefs is possible but may be limited to some degree by adaptations of deep-water species to increased light levels and other environmental factors. Also, the range of species which could colonize shallower depths is probably a small subset of those already living there.

1.3.6 Origin(s) of recolonizing corals

Could damaged reefs be recolonized horizontally rather than vertically, as discussed above (i.e. from other latitudes)? This actually has a high probability of occurring. This is based on the geological and palaeontological records. It would most likely not be a short- or medium-term solution to the problem of replenishing reefs severely affected by increased seawater temperatures, but a long-term one requiring most likely minimally hundreds of years.

There are precedents for this in the geological record. For example, the Caribbean experienced two

major extinctions in the past. During the end of the Oligocene/beginning of the Miocene sea level fell by ~50–75 m (Kominz, 2001) and the Isthmus of Panama was formed, severing the Tethys Sea into the Atlantic and the Pacific Oceans (Stanley, 1979, 1984; Veron, 1986; Rosen, 1988). This change in sea level was accompanied by oceanic cooling in the Atlantic, particularly in the Caribbean, which caused mass extinctions of marine fauna, particularly in bivalves and corals (Wells, 1956). Some of the coral community in South America survived. This region appears to have served as a refuge for coral species during this period as the corals here did not experience the same degree of cooling. Later, when the planet entered a warming phase, the remaining coral species, lower in species diversity, were able to recolonize the Caribbean from South America (J.E.N. Veron, Bali, Indonesia, 2000, personal communication). The Caribbean remained relatively stable after this, as the Earth continued to warm.

This series of events was repeated. At the end of the Pliocene/beginning of the Pleistocene, the Earth experienced another cooling period characterized by a major glaciation (Stanley, 1979, 1981, 1984, 1985, 1986). Again, there were mass extinctions of bivalves and corals in the Caribbean (Dana, 1975; Frost, 1977). In time, the climate changed again and the glaciers melted and receded. The Caribbean waters warmed, making it suitable for expansion of coral populations back into the Caribbean region again to re-establish communities there. It is possible that something like this may happen again, although clearly it will require long periods of time.

1.3.7 Will temperate azooxanthellate corals be affected by climate change?

Temperate azooxanthellate corals will most likely not be as affected in the short term by predicted increases in seawater temperatures in the temperate zone as tropical zooxanthellate corals. The reason is that they do not possess zooxanthellae, and it is the zooxanthellae that are the more sensitive of the symbiotic pair to high seawater temperatures – not the coral tissue (Strychar *et al.*, 2005; Strychar and Sammarco, 2009). However, they may be affected in the longer term. In tropical corals, once seawater temperatures reach 32–34°C, the cells within the coral tissue also begin to exhibit signs of apoptosis and necrosis, and the corals begin to die (Strychar *et al.*, 2004; Strychar and Sammarco, 2008). At this

time, we do not know at what temperatures this type of cellular response might occur in temperate corals. This type of response may actually be initiated at temperatures lower than tropical corals, because these corals are generally adapted to live under cooler conditions than tropical and subtropical ones. Based on this potential response, it is likely that, in areas within the temperate region that are exposed to high seawater temperatures, it is possible that we may also lose those coral communities in the future.

1.3.8 The effects of increasing seawater temperatures on coral reproduction

Adult corals are not the only ones that will be affected by increasing seawater temperatures in these marginal temperate environments. Coral embryos and juveniles may also be affected by increased temperatures which are known to act on larval development, larval dispersal, larval settlement and early spat growth/recruitment. As seawater temperatures increase, fertilization of the coral eggs will occur without hindrance (Bassim *et al.*, 2002). Embryonic development of the planular larvae, however, is strongly affected by temperature (Bassim and Sammarco, 2003). That is, with increasing temperature, the larvae develop in an abnormal and teratogenic fashion, making them inviable. If the larvae do develop fully, their swimming capabilities are greatly diminished with increased seawater temperature, as are their settlement capabilities, and spat survival capabilities. Thus, any attempts to artificially increase the survival capabilities of adult corals should take into account survivorship in the planular larvae and the spat.

1.3.9 The effects of heat stress on zooxanthellar clades in coral

Symbiodinium sp. occurs in the form of many clades, each varying in their DNA sequences. These clades thus far number 12–14 (Sammarco and Strychar, 2009), with numerous (~1700) subclades and subtypes being described using various methodologies (see Riddle, 2007). The immune system of the individual clade must produce one or more proteins for recognition by the host coral in order for successful colonization of the host to occur. Likewise, the coral must have the ability to recognize more than one clade's proteins at the same time, and this capability is genetically based.

The possession of, say, three clades requires more genetic variation and metabolic energy than possessing a single clade. Accommodating each clade requires both the genetic code to do so and also, upon demand, the energetic resources for the production of recognition cells. Accepting multiple clades multiplies the demand on genetic material and energetic resources. Heat stress increases the demand on the coral host by producing macrophages to destroy dysfunctional algal cells of one clade while, for example, in a three-clade system, maintaining the other two. Similarly, it is likely that those corals that have multiple clades are more susceptible to heat stress and mortality than those possessing a single clade, because there is less energy available to deal with this stress and other confounding stresses.

Azooxanthellate corals represent the extreme end of this spectrum because they lack zooxanthellae. *T. coccinea* may serve as an example of this point. This species does not possess zooxanthellae, but does possess pigments. The animal tissues of this species will bleach its pigments under heat stress, but not until seawater temperature reaches $> 36^{\circ}\text{C}$ (Strychar *et al.*, unpublished data). This is a very resilient coral and is temperature resistant. Some corals are aposymbiotic, wherein possession of zooxanthellae is facultative. An example of such a coral is *Oculina*, including *Oculina diffusa*, the genus of which occurs throughout the western Atlantic and the Mediterranean. *O. diffusa* has at least two clades of zooxanthellae, B-1 and B-2 (LaJeunesse, 2001, 2002, 2004; Banaszak *et al.*, 2006; Riddle, 2006) and exhibits bleaching under heat stress (Savage *et al.*, 2002) and disease conditions (Kushmaro *et al.*, 1996, 1997, 1998, 2001; Rosenberg *et al.*, 1998; Sutherland *et al.*, 2004; Anonymous, 2009). We would predict that, since accepting a zooxanthellar clade is a presence/absence character, it would exhibit the same level of immunity as a zooxanthellate coral with the same potential number of clades. Likewise, we would expect this species to have similar heat sensitivities or susceptibility to disease as other corals that accept three clades of zooxanthellae. In fact, *Acropora formosa* can accept three clades of zooxanthellae and is listed on the International Union for Conservation of Nature (IUCN) red list of endangered species (Carpenter *et al.*, 2008) because of mass mortalities due to both heat stress and disease. *Oculina varicosa*, known to have only one zooxanthellar clade, is similarly listed (Roberts and Hirshfield, 2004).

1.3.10 Immune responses of coral relative to heat stress

If we assume that bleaching (i.e. heat stress) is tied to the immune system, and that zooxanthellae are maintained within the host by being recognized immunologically as 'self', then the question arises regarding how the number of clades carried by a coral affects susceptibility to bleaching. The most logical and parsimonious answer would be that having more than one clade with differential temperature susceptibilities per clade confers better fitness on the holobiont. This is because of the cumulative broader temperature tolerances between the clades. However, more clades require more immunorecognition proteins to address the presence of benign symbionts. Possession of a single clade may mean that only one protein is required. The system whereby several clades in a single colony enhance survival is driven by simple directional selection and specialization of temperature tolerances. These tolerances may be spread among two or more symbiotic clades, conferring a broader temperature tolerance for the holobiont. The data, however, suggest a different situation. For example, *Acropora hyacinthus* (Great Barrier Reef) can possess up to three clades simultaneously in a colony; and yet, as shown through controlled laboratory experiments, this species is highly susceptible to bleaching with only modest increases in seawater temperatures. It is the most temperature sensitive of three species from three families tested. On the other hand, *Favites complanata* possesses two clades of zooxanthellae and is less susceptible to bleaching than *A. hyacinthus*. Further, *Porites solidia* has only one clade of zooxanthellae and is the most resistant to bleaching and least sensitive to elevated temperatures. Furthermore, the azooxanthellate coral *T. coccinea*, possessing no zooxanthellae, exhibits no signs of heat stress up to temperatures of 36°C , at which point the coral begins to 'bleach' its tissue pigments and eventually dies. In this case, the immune system need only recognize 'self' cells, as there is no need to allow other symbiotic cell types to enter the organism. This would imply that necessity for a relatively sophisticated immune system is diminished in a symbiotic coral, whereas in azooxanthellate coral, immunity is focused on self-defence. We hypothesize that this is because the host, when it invests energy into defence, directs all of it into a single macrophagic type; metabolic energy is not allocated across the production of

numerous macrophage types or shared among several types of symbiotic algae. This allows the azooxanthellate coral to respond to dysfunctional cells or infection rapidly.

Once again, these results may seem counter-intuitive. Why would the presence of the second clade which has temperature sensitivities different from its counterpart not increase the probability of survival of the holobiont (i.e. the coral host)? The reason, again is most likely related to immunity. In a coral with zooxanthellae, whether it possesses one clade, two, or more, when temperature is elevated, the zooxanthellae, which had been benign symbionts recognized by the coral as 'self', now experience a change in their cell membrane structure and become recognized as 'non-self'. As 'non-self', they are essentially treated the same as a pathogen. In a zooxanthellate coral there can be up to tens to hundreds of millions of zooxanthellae spread throughout the organism. When the cell surface signal changes to communicate a 'non-self' signal to the host coral organism, this occurs throughout the zooxanthellar population nearly simultaneously. This then changes the existing symbiont population into the equivalent of a massive pathogenic infection almost simultaneously throughout the colony, somewhat analogous to leukaemia in humans. Those red blood cells, critical to the survival of the individual, are treated as pathogens by the immune system/white blood cells – by the hundreds of millions. This then requires a tremendous amount of energy to fight off the infestation of millions of non-self entities already omnipresent, now perceived to be pathogenic cells. This is complicated by a concomitant reduction in energy resources for the coral, in the form of carbohydrates which the zooxanthellae had been supplying to the host. This could stretch the coral to its physiological limit. As the zooxanthellae die, less and less food would be available to provide the energy to feed the host – to produce and maintain the macrophages to control the pathogens.

1.3.11 Immune response as the primary process driving bleaching: a hypothesis

How is the signal of 'self' to 'non-self' manifested in the zooxanthellae under heat-stress conditions? We propose that there may be a conformational change in the surface proteins produced by *Symbiodinium*. This change may be the basis for

the lack of recognition of 'self' for the algae by the coral. If so, it would trigger an immune response on the part of the host. That is, it may not be the apoptosis and necrosis in the *Symbiodinium* which initially causes the breakdown in the host-symbiont link; it may be the breakdown in the immunorecognition system required for this successful symbiotic relationship. Apoptosis and necrosis would then follow after lack of recognition and attack by macrophages. In a recent study, one of us (Strychar, unpublished data) was able to differentiate macrophage types using morphology via transmission electron microscopy, following the techniques of Tahseen (2009). The processes of apoptosis and necrosis are not the cause of bleaching; they are symptoms of lack of immunorecognition as 'self' and represent precursors to bleaching.

Communication between the host and symbiont, or the breakdown thereof, may well be regulated by the immune system. The caspases may play a particularly important role here. Upon injury of chemical or heat stress, cell membrane conformational changes would result in the release of caspase markers, which would mark the injured cells as dysfunctional and attract macrophages for the purpose of removal. The macrophages are responsible for causing cellular destruction, which is then observed as apoptosis and necrosis. This has been repeatedly documented at the cellular and subcellular level.

This same type of immunorecognition would most likely occur in response to a number of environmental perturbations, including changes in ocean temperatures, ocean acidification, sea level rise, etc. The driving factor would be the same in all cases, most likely a change in cell membrane conformation. A change in the molecular structure or configuration in the cell membrane may serve as an important cue for macrophage attack.

Cervino *et al.* (2012) has demonstrated experimentally that a coral infected with the bacterial pathogen *Vibrio* sp. continued to suffer for 10 years until death. This suggests that once a coral is infected with such a pathogen, it may be controlled to a degree, but not destroyed; rather, it persists long term until eventual death of the colony. This is unlike heat stress. The disease is a constant presence and the battle continues within the coral; the energy supply of the coral is depleted through a chronic production of macrophages attempting to control the disease. This is analogous to AIDS (acquired immune deficiency

syndrome) in humans – a chronic infection which suppresses the immune system, opening the host to secondary infections, which can be fatal. Bleaching is an acute response to heat perturbation; disease is a chronic response to a pathogenic perturbation. We raise the question, are diseased corals compromised to a greater degree than temperature-bleached corals because the probability of recovery may be lower due to a potential chronic infection? If so, can we expect the immune response to vary species-specifically for the coral and the disease, respectively? Alternatively, perhaps the query should be the other way around. Are corals stressed due to high temperature under pre-bleaching or bleaching conditions and compromised allowing infection by disease? And do they have a lower probability of recovery due to infection?

1.3.12 Evolution of zooxanthellate versus azooxanthellate corals

Azooxanthellate corals appear to have broader environmental tolerances than zooxanthellate ones. They are not depth-restricted because of light. They also have broader temperature tolerances. They are heterotrophs. Increases in nutrient enrichment are often accompanied by increases in plankton populations, which can serve as a stress for zooxanthellate corals but as additional food sources for the azooxanthellate corals. A comparison between azooxanthellate and zooxanthellate corals may be somewhat analogous to a comparison between horseshoe crabs and fruit flies, respectively. Horseshoe crabs (*Tachypleus tridentatus*) are an extremely old taxon, dating back in their current morphological form to the late Ordovician, approximately 450 million years ago (MYA) (Rudkin *et al.*, 2008). Their physiological tolerances are very high with respect to variations in temperature, salinity, oxygen concentrations, etc. (Sekiguchi and Shuster, 2009). On the other hand, *Drosophila* spp. (fruit flies) have a very high genetic variability and have speciated numerous times. Currently 1450 species of *Drosophila* are documented to occur naturally. The genetic variability in this genus is formidable, and the physiological and environmental tolerances of each species are very narrow (Dobzhansky, 1970; Steiner, 1977).

Zooxanthellate corals are more species diverse than the azooxanthellate ones – at the generic and family levels, in the Caribbean and the Indo-Pacific (Veron, 1996, 2000). Azooxanthellate corals

preceded zooxanthellate ones in evolutionary time. It has been proposed that scleractinian corals evolved 225 MYA during the mid-Triassic (Romano and Palumbi, 1996), and endosymbiosis with zooxanthellae evolved 200 MYA during the late-Triassic (Stanley and Swart, 1995; Stanley and van de Schootbrugge, 2009). Azooxanthellate corals appear to have had less adaptive radiation than their zooxanthellate counterparts, making them more evolutionarily conservative. For example, the zooxanthellate coral genus *Acropora* (Acroporidae) is known to have arisen about 60 MYA during the Paleocene (Carbone *et al.*, 1994) or 45 MYA during the Eocene (von Fritsch, 1875; Latham, 1929), and currently has as many as 180 described species (Veron, 1996), mostly in the Indo-Pacific. One of the reasons for their broad phylogenetic radiation may be derived from their mode of reproduction (simultaneous multi-species broadcast spawning of sperm and eggs into the water column) and their propensity for hybridization (Willis *et al.*, 2006). The genus *Tubastraea* (Dendrophylliidae) is a brooder and, similar to *Acropora*, apparently emerged during the early Eocene, 55 MYA. Yet *Tubastraea* is known to have only eight species, two of which are extinct (Cairns, 2001). This genus is approximately 10 million years older than *Acropora*. The major point is that azooxanthellate corals may be the older, more conservative, and less specific group in terms of environmental limitations yet have retained the ability to disallow colonization by foreign symbionts by possessing more caspases.

1.4 Climate Change/Global Warming and the Long-term CO₂ Problem

The problems we face with climate change and global warming will not be solved in the short term. This is because the amount of: (i) atmospheric CO₂ is already massive; (ii) heat this has created secondarily in our atmosphere over the past 150 years is elevated; and (iii) heat which has, in turn, been introduced into our oceans is increasing. It has been estimated that $> 25 \times 10^{22}$ KJ of heat have been absorbed into the oceans since 1957 as a by-product of CO₂ release (Church *et al.* 2011). The ocean is a large heat sink. Water also has a high thermal capacity, which means it is slow to warm and slow to cool. Even if we stop introducing CO₂ into the atmosphere today, it will be 2075 before the oceans will begin to cool.

The production of atmospheric CO₂ will continue for quite some time, on an ecological timescale. Some special-interest groups place blame on certain groups for the excessive CO₂ released into the atmosphere, as well as for the continuance of this problem. The truth is that we are all to blame. Over the 20th century, humans have become dependent upon, indeed addicted to, the use of petroleum hydrocarbons for transportation and production of numerous everyday products. It is likely that their use cannot – and will not – be curtailed in the short term. Any decline in usage of this non-renewable, non-sustainable natural resource will require a coordinated and concerted effort on the part of all stakeholders – users and producers – to reduce usage and substitute suitable renewable, sustainable resources for the hydrocarbons. This would then result in a gradual decline in petroleum hydrocarbon usage. It is our prediction that those oil and gas companies that are willing to invest a substantial proportion of their profits now into research on renewable, sustainable energy sources will most likely not only survive this changeover in energy sources in the future, but also dominate energy production. The sooner this corporate tack is adopted, the sooner our environment will begin to recover, and the sooner those companies will develop a competitive edge over other companies in their sector.

1.5 Closing Comments

Will the temperate zone play a limiting role in the changes in the distribution of tropical and subtropical corals and the reefs they build? The answer is, yes. They will do this by limiting the amount of light available for photosynthesis by the coral's zooxanthellae, including through changes in length of daylight. They will also limit the quality of the light made available to the corals due to changes in the angle of the sun's light impacting the corals and concomitant changes in the light spectrum impacting the benthos. In addition, these zones will limit the temperatures in which the tropical corals will be able to survive or build calcium-carbonate-based reefs. Temperate corals are already adapting to these cooler temperatures and are not dependent upon light for photosynthesis for survival. They will continue to play their role of a recognizable component of the benthic community. They will not, however, take over the role of building coral reefs; they do not have the physiology to do it.

As coral reefs go through changes with time through mortality of the more sensitive species, recolonization may come from deeper mesophotic reefs, but only to a degree. In the longer geological timescale, it is more likely that recolonization of the Caribbean will come from refuges in other higher latitude countries, such as Brazil, which may be less affected by increases in seawater temperature, as has apparently happened before in geological history.

Is it possible for us to address the problem of oceanic warming today? Yes. Anything we do to reduce CO₂ emissions will contribute to this effort. But the effort must come from all stakeholders, and it will need to be long term – perhaps over several generations. This is because, after the effort has been initiated, it will still require years for the oceans to cool and begin to reverse the ecological damage that we are now witnessing. It is time for us to become good stewards of our environment, and then pass that torch down to our children and grandchildren.

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2

Tropical Marine and Brackish Ecosystems

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2.1 Climate Change and the Tropics

The tropical region delimited by the Tropics of Cancer (23°N) and Capricorn (23°S), receives direct solar radiation throughout the year. Its water is warm (mean average sea surface temperatures (SSTs) of > 24°C) with high salinity and, depending on location, can be nutrient poor to rich. These conditions promote primary productivity and are ideal for the formation of several important and highly biodiverse marine and brackish water ecosystems, such as coral reefs, seagrasses and mangroves (Fig. 2.1). These ecosystems support the highest species diversity and gross productivity that generates goods and services (e.g. food, fisheries, coastal protection, tourism, natural products) which directly or indirectly support millions of people. Despite their importance, these sensitive ecosystems are increasingly under pressure from multiple, concurrent stressors that severely compromise their health and resilience, reducing and risking the loss of their capacity (Folke *et al.*, 2004; Waycott *et al.*, 2009; Spalding, 2010). Coral reef, seagrass and mangrove ecosystems have declined rapidly in the last 50 years, and are deteriorating due to human activities such as increased coastal development, pollution, sedimentation, eutrophication, diseases, as well as irresponsible fishing and tourism (Wilkinson, 1996; Barker and Roberts, 2004; Fabricius, 2004; Saphier and Hoffmann, 2005; Erftemeijer and Lewis, 2006;

Polidoro *et al.*, 2010; Friess *et al.*, 2016). Concurrently, the detrimental impacts of environmental disturbances related to climate change are becoming progressively apparent and alarming in recent years (Hughes *et al.*, 2017a, b; Nowicki *et al.*, 2017).

Climate change in this chapter refers to the warming of Earth in response to human activities, which have directly and indirectly increased the concentrations of atmospheric heat-trapping gases (or 'greenhouse' gases) such as carbon dioxide (CO₂), nitrous oxide (N₂O), methane (CH₄), and other compounds such as chlorofluorocarbons (CFCs) (World Bank, 2013; IPCC, 2014). While the climate is naturally variable and can fluctuate (in human lifetimes) on timescales of 10–100s of years, these increases in greenhouse gas concentrations go beyond natural climate fluctuations and are brought about mainly by human intervention through land use changes and burning of fossil fuels (IPCC, 2014). The impacts of climate change on the global environment have been examined based on different emission scenarios (see IPCC, 2014). Predicted large-scale long-term effects of climate change in tropical regions include changing weather patterns, increase in storm surges, sea warming, rising sea level, ocean acidification, reduced ocean circulation and changes to ocean salinity and oxygenation (Rhein *et al.*, 2013; IPCC, 2014).

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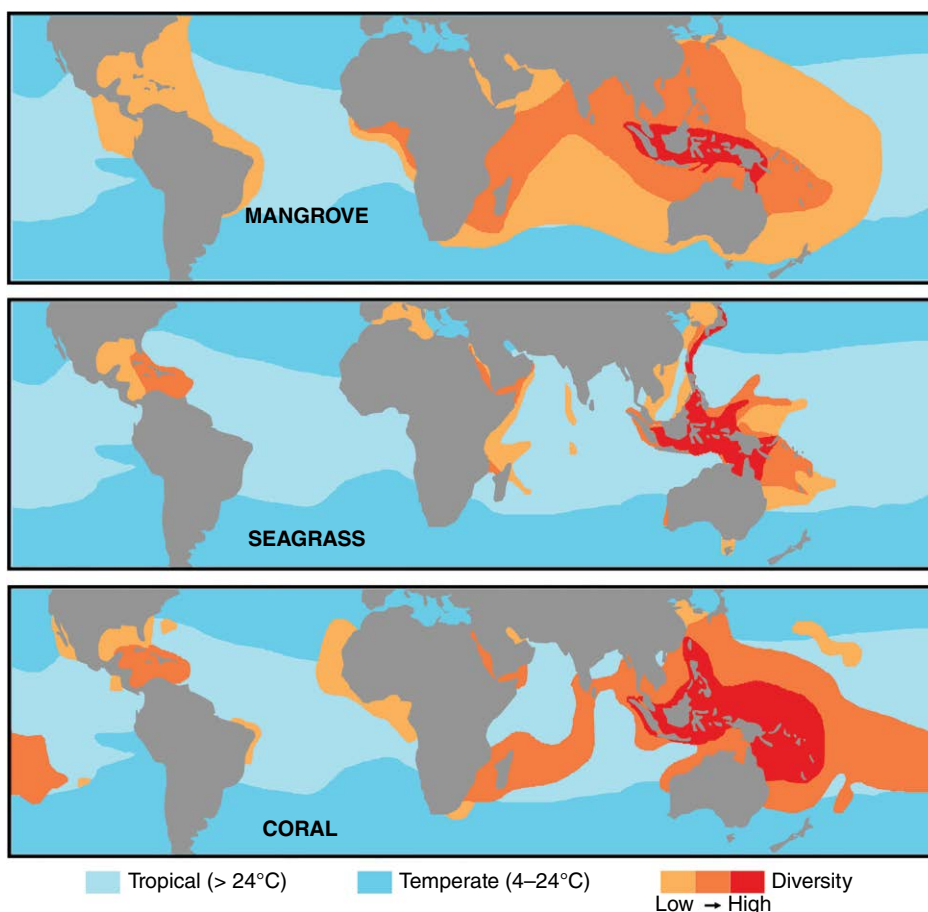


Fig. 2.1. Global mangrove, seagrass and coral distribution in relation to mean sea temperature. Regional divisions are based on tropical (> 24°C) and temperate (4–24°C). (Adapted from Wells, 2006.)

There are, however, vast differences and uncertainties in the extent of model-projected climate changes. These can be attributed to several factors such as the climate model (both global and regional) dynamics, spatial resolutions of these models (including the difficulty in downscaling), inadequate understanding of atmospheric and oceanic processes that lead to limitations in modelling and different assumptions of climate scenarios. These cascades of uncertainties lead to a need for a better quantification and understanding of climate projections. Especially, when the results of the climate models are used as inputs for impact models, these uncertainties propagate further, thus augmenting the uncertainties in climate projections (Mearns *et al.*, 2001). However, despite such uncertainties, the evidence linking impacts of climate change on

biological, physical and human systems has never been more compelling, in part because of improved reporting of published studies from under-represented regions of the world especially in the tropics (Rosenzweig and Neofotis, 2013).

In this chapter, we present updates on current conditions and expected changes on key abiotic parameters (i.e. temperature, rainfall, circulation, seawater carbonate chemistry, sea level, salinity, oxygen, nutrients) and the range of predicted impacts on key tropical marine and brackish water ecosystems, namely coral reefs, seagrasses and mangroves.

2.1.1 Rising temperatures

While rates of warming vary between regions, there is clear evidence that surface air and seawater

temperatures in the tropics have risen significantly in recent decades: average surface air temperatures increased from 0.1°C to 0.3°C per decade (1950–2010) and seawater temperatures (< 75 m depth) by 0.11°C per decade from 1971–2010 (Rhein *et al.*, 2013; IPCC, 2014). By the end of the 21st century, it is estimated that sea temperatures (< 100 m depth) will rise anywhere between 0.6°C and 2.0°C. Sea surface warming is predicted to be most pronounced for subtropical and tropical regions under all representative concentration pathways (RCP) scenarios (IPCC, 2014). However, there is large spatial variability in the rates of warming across the tropics (e.g. warming of the Andaman Sea is currently twice the rate of that for the South China Sea) (Xie *et al.*, 2010; Collins *et al.*, 2013; Tanzil *et al.*, 2013; IPCC, 2014). Patterns of warming in the tropics are generally less well-documented and not as systematically studied (as the global mean). This is because spatial variations in surface warming within the tropics are considerably smaller, making them hard to discern on a global map. Spatially downscaled climate models are therefore extremely important to improve accuracy of warming predictions for tropical seas.

An oceanographic region of extreme importance for the tropics is the Indo-Pacific Warm Pool (IPWP) (Weller *et al.*, 2016). The IPWP, which comprises the Indian Ocean Warm Pool and the Western Pacific Warm Pool, is the Earth's largest region of warm SSTs which exceed 28°C (an estimated threshold for atmospheric deep convection). The IPWP has warmed and grown substantially during the past century (Weller *et al.*, 2016). As the IPWP is considered fundamental to global atmospheric circulation and hydrological cycle, changes/fluctuations to the IPWP intensity and size are predicted to affect rainfall distribution and sea level rise throughout the tropics (Williams and Funk, 2011; Weller *et al.*, 2016). Already the IPWP has experienced the world's highest rates of sea level rise in recent decades, indicating large increases in ocean heat content. Due to its proximity to areas with large marine biodiversity (Fig. 2.1), further increases in SST in the IPWP are therefore expected to have serious ecological consequences (De Deckker, 2016).

2.1.2 Changes in rainfall and tropical storm patterns

Over 40% of the global precipitation falls within 15° of the equator, and the seasonal north–south

movements of the Inter-tropical Convergence Zone (ITCZ), where moisture-laden trade winds converge on the warmest regions of the ocean, regulate precipitation patterns and cloud cover throughout the tropics. There has been a significant narrowing of the ITCZ in recent decades, with future annual-mean narrowing predicted due to global warming (IPCC, 2014; Bryne and Schneider, 2016; Wodzicki and Rapp, 2016). Furthermore, over the decades the southern edge of the Pacific ITCZ has migrated farther northwards than the northern edge southwards (Wodzicki and Rapp, 2016). This narrowing and shifting of the ITCZ could greatly affect large-scale rainfall distribution. While there are still huge uncertainties for projections of rainfall with global warming, especially for the tropics, the premise that while mean precipitation remains largely unchanged, wetter areas and seasons are likely to get wetter, and dryer areas/seasons drier appears to hold (Fig. 2.2; IPCC, 2014). Relative change in local SSTs is a major driver of spatial variability in rainfall patterns: in many parts of tropical oceans, convection is reduced despite an increase in local SST because the local warming falls below the tropical average. However, in other parts of the tropics where relative SST change is positive, precipitation generally increases. The warmer-get-wetter pattern dominates in coupled atmosphere-ocean models and deviates from the general 'wet-get-wetter' pattern realized in atmospheric response to uniform SST increase (Ma and Xie, 2013; Chen and Bordoni, 2016). Spatial variability in projection of rainfall patterns is also compounded by impacts of disturbances to global circulation systems (e.g. El-Niño Southern Oscillation (ENSO), Indian Ocean Dipole (IOD), monsoons), which further increases prediction uncertainties, especially for small tropical islands (World Bank, 2013; IPCC, 2014). Increase in frequency of rainfall extremes is predicted to lead to increases in flood frequency and/or increases in drought days in the tropics.

While storm frequency is predicted to remain unchanged, or even decrease, projections into the 21st century show an increase in intensity with regard to maximum wind speed and rainfall rates (Villarini and Vecchi, 2013; IPCC, 2014). As to rainfall patterns, the influence of future climate change on tropical storms and cyclones are likely to vary from region to region, with low confidences in region-specific projections attributable to compounded uncertainties from predictions of other climate variables that influence regional tropical storm activity.

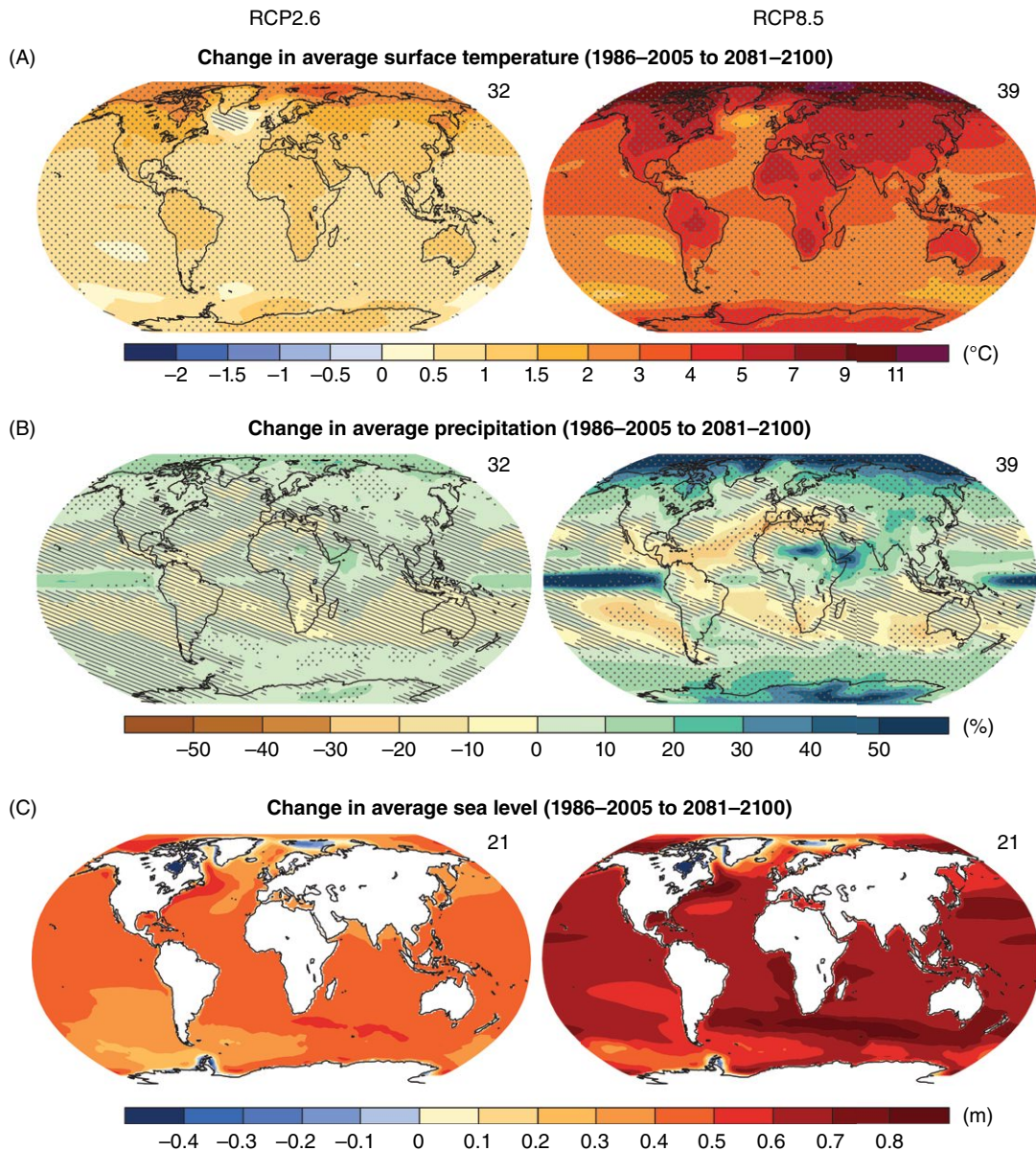


Fig. 2.2. Change in average surface temperature (A), change in average precipitation (B) and change in average sea level (C) based on multi-model mean projections for 2081–2100 relative to 1986–2005 under the representative concentration pathway (RCP)2.6 (left) and RCP8.5 (right) scenarios. (From IPCC, 2014.)

2.1.3 Ocean and atmospheric circulation systems

Changes in atmospheric and oceanic circulations are a means for the Earth to self-regulate its climate, which often affects wind patterns and pressure fields over different parts of the world. In the

tropics, the ENSO, which relates to changes to the Walker circulation, will likely remain as the dominant mode of coupled ocean-atmosphere climate variability in the 21st century (IPCC, 2014). ENSO refers to the periodic variation between positive and negative SST anomalies, and how they impact

dry and wet conditions over the following years. During a neutral ENSO phase, surface trade winds are easterly across the Pacific Ocean resulting in a westward surface ocean current. When ENSO is in an El-Niño phase, the surface ocean warms as western winds weaken, sometimes changing direction. La-Niña years have below average SSTs in the central and eastern Pacific, so that the ocean surface cools as easterly winds strengthen. Generally, ENSO occurs irregularly with a 4–7 year interval, but is predicted to further increase in frequency and intensity with global warming (IPCC, 2014). With changes to ENSO, large variability in regional-scale rainfall and fluctuations in sea temperatures are expected. As it is, anomalous sea warming events associated with the El-Niño phase during strong ENSO years (e.g. 1998, 2010, 2016) have already caused devastating impacts for marine and brackish ecosystems across the tropics (e.g. Hughes *et al.*, 2017b; Nowicki *et al.*, 2017). Similar oscillations in SSTs also occur in the tropical Indian Ocean. Another teleconnection that influences tropical rainfall is the IOD. While less studied than ENSO, environmental anomalies driven by the IOD and/or its interaction with ENSO have similar predicted consequences for ecosystems in the Indian Ocean (Zhang *et al.*, 2017).

Monsoon systems are also dominant drivers of seasonal climate variation in the tropics (e.g. East Asian Winter Monsoon, African Monsoon, East Asian Summer Monsoon). Monsoon systems are important, as they bring most of the annual rainfall in many tropical regions. Driven primarily by land-sea temperature contrasts, which vary seasonally with the distribution of solar heating, monsoon timing and strength are also predicted to change with future warming trends. Disturbances to monsoon systems have been linked to ENSO variations but the impacts are complex (e.g. Feng *et al.*, 2014; IPCC, 2014; Roy *et al.*, 2016; Xue and Zhao, 2017). It is predicted that while overall monsoon circulation may weaken, the global monsoon precipitation is likely to strengthen in the 21st century, increasing in both area and intensity that will result in increased monsoon-related flooding. Changes in seasonal monsoon onset dates, and monsoon retreat dates, are also expected with further warming and disturbances to climate modes (e.g. ENSO, IOD).

Ocean currents and circulation are extremely important in the transport and redistribution of heat, carbon and nutrients the Earth's oceans sequester. Ocean circulation can be conceptually

divided into two components: (i) a relatively fast-moving wind-driven circulation that occurs near the ocean surface; and (ii) a slow density-driven circulation that displaces oceanic water in both horizontal and vertical directions. In the tropical zone of the Pacific and Atlantic Oceans, the surface water is relatively warm and driven westwards by easterly winds. Upwelling of cold and nutrient-rich water to the surface occurs in the east of the ocean basin close to the equator. This results in an equatorial cold tongue that spans from the east boundary to the middle of the ocean basin and surrounded by a warm pool of water in the north and south. In contrast, the tropical Indian Ocean is mainly filled with warm water at the surface with an upwelling zone located in the south-western part of the basin (Chen *et al.*, 2015).

Climate change is expected to weaken the strengths of several important oceanic currents which influence tropical marine and brackish water ecosystems. One such current is the Indonesian Throughflow (ITF) (Fig. 2.3). The ITF transports a large amount of heat and fresh water from the Pacific Ocean to the Indian Ocean; it cools the Pacific and warms the Indian Ocean (Hirst and Godfrey, 1993; Godfrey, 1996). There are two pathways by which ocean water moves with the ITF: one originates from the North Pacific Ocean and the other transports water from the South Pacific Ocean. The dominant pathway is driven by the Mindanao Current that continuously penetrates the Celebes Sea with water derived from the North Pacific Ocean, which then moves through the Makassar Strait towards the south into the Flores and Banda Seas and finally exits through the Lombok Strait, Ombai Strait and Timor Passage into the South-western Indian Ocean. The second pathway moves deeper, more saline water derived from the South Pacific Ocean along an eastern route from Maluku to Seram to the Banda Seas where it joins the North Pacific Ocean water from the first pathway before exiting into the Indian Ocean (Gordon and Fine, 1996; van Aken *et al.*, 2009). Meyers (1996) demonstrated that the ITF is sensitive to the ENSO: the ITF is stronger during La Niña and weaker during El Niño. On a centennial timescale, climate models consistently project a substantial weakening of the ITF in response to climate change (Hu *et al.*, 2015; Sen Gupta *et al.*, 2016). Feng *et al.* (2017) suggest that the weakening of the ITF transport in a future warmer climate will result from a reduction in the rate of deep-water

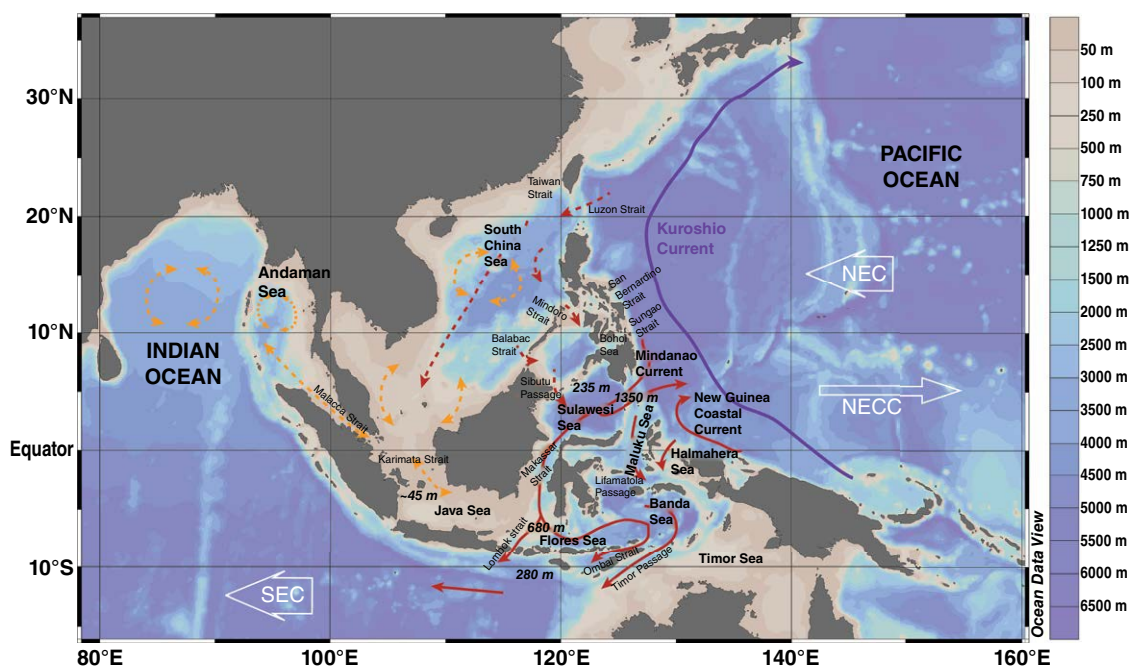


Fig. 2.3. Schematic of the South China Sea Throughflow (dashed red arrows), the Indonesian Throughflow (solid red arrows) circulations and reversing monsoon currents (dashed orange arrows). NEC, North Equatorial Current; NECC, North Equatorial Counter Current; SEC, South Equatorial Current.

formation in the Southern Ocean and stronger stratification of the water column caused by a slowdown of upwelling from the deep Pacific Ocean. Changes to the ITF will not only effect the heat flux between oceanic basins, but also the environment in its pathways, especially in the Maritime Continent, which extends through the world's marine biodiversity hotspot (Fig. 2.1).

Less known and understudied, but likely to be important to the Maritime Continent, is the South China Sea Throughflow (SCSTF), which has its main inflow through the Luzon Strait and outflows via the Karimata, Mindoro and Taiwan straits (Fig. 2.3). The SCSTF acts as a heat and freshwater conveyor, playing a potential important role in regulating the SST pattern in the South China Sea and its adjoining tropical Indian and Pacific Ocean (Qu *et al.*, 2006). The Kuroshio Current drives the pathway of SCSTF through the Luzon Strait in the western North Pacific Ocean as it moves northwards. As the South China Sea is a recipient of heavy rainfall, the surface water of the SCSTF is freshened as it moves across the basin (Qu *et al.*, 2009). Water then exits the South China Sea basin

through two pathways – one via the Mindoro Strait into the Sulawesi Sea, and another via the Karimata Strait into the Java Sea. The shallow Karimata Strait pathway of SCSTF brings water from the South China Sea into the Makassar Strait in the upper 45 m layer and is mainly dependent on local monsoon wind (Gordon *et al.*, 2012). The Mindoro Strait pathway of SCSTF impacts the ITF, especially during El Niño events (Tozuka *et al.*, 2009; Gordon *et al.*, 2012). As mentioned, the ITF is weaker during El Niño and this is globally balanced by the SCSTF which intensifies during El Niño events due to higher flow from the western Pacific Ocean through the Luzon Strait which induces an increase of southwards flow of buoyant surface water into the Sulawesi Sea via the Mindoro Strait. This intensification of the SCSTF inhibits tropical Pacific surface water injection into the Makassar Strait. During La Niña, the impact of the SCSTF is minimal which allows inflow from the tropical Pacific that intensifies the ITF.

Another important ocean circulation process that climate models predict will weaken over the 21st century and impact the tropics is the Atlantic

Meridional Overturning Circulation (AMOC) (Cheng *et al.*, 2013). The AMOC delivers warm surface water from the tropics and southern hemisphere towards Greenland in the North Atlantic, which then sinks as it cools and flows back towards the equator closer to the seafloor. According to Liu *et al.* (2017) the AMOC is in an unstable regime susceptible to large changes in response to perturbations, whereas Collins *et al.* (2013) anticipated a more gradual weakening of AMOC over the decades. Nevertheless, scientists agree that the collapse of AMOC will have repercussions on the entire global ocean circulation and studies continue to elucidate the nature and extent of these impacts.

2.1.4 Rising sea level

Global mean sea levels rose at 1.7 mm/year throughout the 20th century, and 3.2 mm/year between 1992 and 2012 (Meyssignac and Cazenave, 2012). Sea level increases are linked to two processes: (i) thermal expansion; and (ii) glacial melting. There is large spatial variability in rates of sea level rise thus far, and in future projections. In the Indian Ocean, sea level increases have been twice that of the average global rates since 2003, a trend projected as likely to continue (Thompson *et al.*, 2016). In contrast, the rate of sea level rise in the South China Sea, western Pacific Ocean is expected to remain similar to the global mean (Huang and Qiao, 2015). The primary cause of such variability is a thermosteric one; differences in rates of thermal expansion related to sea warming drives the rates of sea level rise. Therefore, for regions that sit between large water bodies warming at different rates, such as South-east Asia (Fig. 2.1), there exist large uncertainties in sea level predictions and its impacts on coastal systems. Additionally, vastly different rises in local sea levels are likely to have a large impact on the strength and direction of the seasonal currents, that are also influenced by monsoonal pressures on the different seas (Tay *et al.*, 2016). Such complex interlinked mechanisms all add further uncertainties to tropical sea level predictions but are currently relatively understudied.

Along with higher intensity tropical storms projections are also increases in tropical storm frequency (see Section 2.1.2), and associated sea level change. Currently, even in relatively sheltered seas and coastal regions, storms can cause sea level to surge up by 0.8 m depending on storm duration and strength (Kurniawan *et al.*, 2015). Combined

with tidal inundation, especially during high spring tides, storm surges can result in severe flooding of low-lying coastal areas – affecting coastal ecosystems and potentially triggering releases of large sediment loads, pollutants and nutrients into the coastal environment.

2.1.5 Seawater carbonate chemistry: pH and carbonate saturation

While sequestration of atmospheric CO₂ by the oceans will help to moderate future climatic changes, it reduces the pH of seawater as CO₂ dissolves to form carbonic acid (Rhein *et al.*, 2013). The current average seawater pH of ~8.1 is already 0.1 units lower than pre-industrial values, which corresponds to a 26% increase in hydrogen ion concentration (Orr *et al.*, 2005; Feely *et al.*, 2009; Rhein *et al.*, 2013). The latest projections from the Coupled Model Intercomparison Project Phase 5 (CMIP5) Earth System models project a reduction in global-mean surface pH that range from 0.06 to as much as 0.32 units by 2100. As pH units are recorded on a logarithmic scale, an increase in 0.32 units represents an increase in ocean acidity of three orders of magnitude (Ciais *et al.*, 2013). The shift in the seawater carbonate system due to increased hydrolysis of CO₂ also drives a decrease in the concentration of marine carbonates, and consequently calcium carbonate saturation levels (Ω). Ω is a measure of the ion activity product of calcium (Ca²⁺) and carbonate (CO₃²⁻) relative to the apparent solubility product for a particular calcium carbonate mineral phase (i.e. calcite, high magnesium (Mg)-calcite, or aragonite). Saturation levels of marine carbonates are thought to have decreased by 10% compared with that of pre-industrial levels (Orr *et al.* 2005), with ~40% further decrease estimated by 2100 (Kleypas *et al.*, 1999; IPCC, 2014). These projected decreases in Ω are expected to have huge impacts on marine calcifying organisms as lower Ω make biogenic calcium carbonate (CaCO₃) more difficult to form (see Section 2.2.3).

2.1.6 Sea surface salinity

Salinity, the weight of dissolved salts per kilogram of seawater, changes according to the addition or removal of fresh water (derived from direct precipitation or via runoff from the land). Salinity is a major factor determining the distribution and composition of marine organisms. Salinity, along with

temperature, also drives stratification in the ocean's mixed layer. Changes in salinity will likely impact both coastal and oceanic tropical marine ecosystems. In the open ocean, the horizontal distribution of salinity in the upper ocean largely depends on the spatial distribution of evaporation and precipitation, with high surface salinity generally found in regions where evaporation exceeds precipitation, and low salinity found in regions of excess precipitation and runoff. Geographical contrasts exist in sea surface salinity: more saline surface waters are found in the evaporation-dominated mid-latitudes, while relatively fresh surface waters in the rainfall-dominated tropical domains. Since the 1950s, climate trends have increased the differences in sea surface salinities between mid-latitude (becoming more saline) and tropical waters (freshening) (Rhein *et al.*, 2013). Climate models predict that there will be +0.5 psu salinity baseline increase in the tropical Atlantic Ocean, and decrease of about -0.5 psu in the western tropical Pacific Ocean by the end of 21st century (Collins *et al.*, 2013; Kirtman *et al.*, 2013).

In addition to direct precipitation, sea surface salinity will also be affected by changing terrestrial, land-based freshwater runoff amounts delivered via rivers, streams, groundwater seepages or overland (derived from rainfall or glacial meltwaters). Milliman and Farnsworth (2011) have estimated from their database that the large rivers (> 400 km³/year flow rate) account for 35% of the freshwater discharge into the coastal oceans. Of these the vast majority are in the tropical belt. Across the tropics, there are no generalized predictions for how riverine inputs will be altered by climatic changes – large spatial variability exists, with both increases and decreases in runoff amounts expected to occur in different areas (Hartmann *et al.*, 2013), related to prediction uncertainties for rainfall patterns (as discussed in Section 2.1.2). However, working with the premise that wetter areas are likely to get wetter, and dryer areas drier, volumes of runoff entering tropical waters will consequently increase in wetter areas and become less in drier areas (Kirtman *et al.*, 2013). Local environments (e.g. land geomorphology, atmospheric moisture content) as well as human modification of the water cycle through use and storage will also determine future volumes of fresh water entering the sea from land. A downward trend in the total volume of freshwater runoff is also observed, associated with human activities and urbanization of the

coastal zones in the tropics (IPCC, 2014). If this trend remains, estuarine balances in wet tropical areas predicted to get wetter could remain unchanged on average, while dry tropical systems will become increasing more saline. However, predicted increases in the frequency of high-intensity convective storms, combined with increased urbanization (IPCC, 2014) are also likely to increase short-term bursts of freshwater flooding (flash floods). These could lead to increased frequency of acute hyposaline events in the future – potentially increasing the range of salinities experienced in coastal environments.

2.1.7 Seawater oxygenation

The concentrations of dissolved oxygen in the ocean thermocline have generally decreased since 1960 (Rhein *et al.*, 2013). This long-term deoxygenation of the open ocean thermocline is consistent with the reduction of oxygen solubility in warmer waters, and a decrease in transport of oxygen from surface to subsurface waters due to warming-induced stratification (Matear and Hirst, 2003; Deutsch *et al.*, 2005; Frölicher *et al.*, 2009). The latest projections from the CMIP5 Earth System models indicate that there will be reductions in mean dissolved oxygen concentrations from 1.5% to 4% (2.5–6.5 $\mu\text{mol/kg}$) in the 2090s relative to the 1990s (Ciais *et al.*, 2013). This will have implications for nutrient and carbon cycling as well as coastal and marine productivity, all of which are dependent on organisms that require aerobic respiration, even in the deep ocean (Keeling *et al.*, 2010). In the absence of well-aerated ocean waters, the continual consumption of oxygen by deep-dwelling organisms could result in the deepest regions of the ocean being devoid of oxygen within decades (Whitney *et al.*, 2007). For mobile organisms, there is probably the ability to seek, find and cope with lowered oxygen levels. However, for sessile organisms or those with restricted movement ranges, lowered oxygen levels could affect respiratory function (e.g. respiration rates in fish is regulated by oxygen levels, unlike humans for which respiration is driven by carbon dioxide levels) (Vaquer-Sunyer and Duarte, 2008), and consequently could bring about possible secondary effects. Oxygen depletion is also likely to become an issue in shallow coastal waters. Where SSTs increase and wind decreases particularly in conjunction with anthropogenic nutrient release,

phytoplankton blooms leading to hypoxic events can lead to increased mass mortality of fishes and other marine life. There are limitations on the resolution to which global ocean models can simulate dissolved oxygen distributions at a local level (Cocco *et al.* 2013), and there is relatively high uncertainty surrounding the modelled projections of evolution of ocean dissolved oxygen and the extent of oxygen 'minimum zones' (Stramma *et al.*, 2012). Regional trajectories of sea oxygenation do therefore require further exploration, refined for local conditions to gain a more comprehensive picture of oxygen depletion in the tropics.

2.1.8 Nutrients

Essential nutrients are required to sustain the ocean's microbiome and primary producers, such as phytoplankton. These primarily are comprised of biologically available forms of nitrogen and phosphorus, as well as silica. Ocean circulation systems (see Section 2.1.3) provide sources of nutrients via vertical (upwelling) and horizontal mixing. While nutrient inputs are vital to support the primary productivity in marine systems, elevated loads could lead to eutrophic conditions. Phytoplankton blooms associated with periods of high nutrient influx (usually in conjunction with other environmental variables such as temperature) result in depleted oxygen and possibly toxic (as in the case of 'harmful algal blooms') conditions for marine life (Wang *et al.*, 2008). For example, harmful algal blooms resulting in massive fish death have been observed throughout the South China Sea related to various regional conditions such as increased upwelling of waters, reversed monsoon winds, eutrophication from coastal aquaculture and river discharges (Susanto and Marra, 2005; Azanza *et al.*, 2008; Wang *et al.*, 2008; Baumgart *et al.*, 2010). As terrestrial runoff not only transports fresh water, but also sediments, nutrients and pollutants (Nicholls *et al.*, 2007), changes to runoff volumes and patterns will not only affect salinity (see Section 2.1.6) but also coastal and estuarine water quality. Furthermore, as nutrient loads to the coastal environment are also a function of land use, it is anticipated that urbanization, without mitigation, will also result in an influx of nutrients. Overall, climatic changes combined with urbanization of the tropical coastal zones are expected to increase eutrophication which will affect coastal ecosystems and fisheries (Sinha *et al.*, 2017).

2.2 Climate Change Impacts on Key Tropical Ecosystems

2.2.1 Mangrove ecosystems

Mangroves occur at coastal intertidal zones between the latitudes of 30° North and South, and are currently estimated to cover about 150,000 km² of tropical (and subtropical) coastline (Tomlinson, 1986; Spalding, 2010). The backbone of mangrove ecosystems are the mangrove trees, which grow best in warm and wet, low wave-energy environments where sediment (from riverine inputs) tend to deposit. Mangrove trees can withstand fluctuating salinities during tidal inundation and, where geomorphology is conducive (e.g. Sundarbans Mangroves) estuarine mangroves can extend several kilometres inland. Favourable environmental conditions encourage the growth of mangrove trees to ~30–40 m. Conversely, dry, cold and low nutrient conditions stunt tree growth (e.g. dwarf mangroves are found in some parts of Central America and Florida). Globally, the biodiversity hotspot for mangrove trees occurs around the Indo-Malay Philippine Archipelago where ~36–46 species are found (Polidoro *et al.*, 2010). This is followed by East Africa with ~14 species (FAO, 2007), and West Africa, the Caribbean, Florida, Atlantic South America, and Pacific North and South America with only 8–10 species (Polidoro *et al.*, 2010).

Mangrove trees employ different strategies to cope with the harsh dynamic intertidal environment (Fig. 2.4): tidal inundation requires them to adapt to fluctuating salinity and waterlogged substrates that are anaerobic as well as unstable (Krauss *et al.*, 2008). The most prominent adaptation of mangrove trees is their elaborate root systems, as exemplified by stilt roots of *Rhizophora* (also called prop roots), knee roots of *Bruguiera* and *Ceriops*, pneumatophores of *Avicennia* and *Sonneratia*, and plank roots of some *Xylocarpus*. These roots often spread laterally to improve support for the trees in the shifting substrate of the intertidal zone, and have numerous lenticels (pores in the woody tissue) to transfer atmospheric oxygen to roots embedded in anaerobic mud (Tomlinson, 1986). To cope with a high salt environment, roots osmoregulate within the root cells and actively exclude salt (Popp *et al.*, 1993), and/or the plants have leaves that secrete salt which are disposed of during leaf senescence. By and large, mangrove species have evolved different tolerance to the inundation conditions, and hence flooding frequency is key to determining tree species establishment and distribution. *Avicennia*

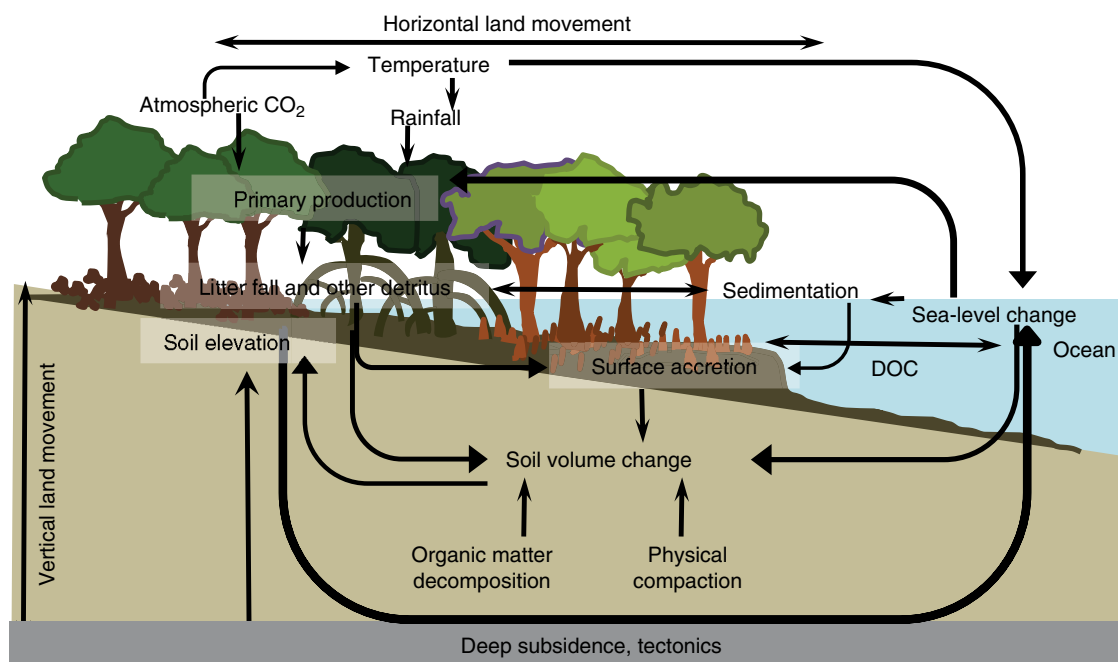


Fig. 2.4. Diagram showing the conceptual interacting physical and biological processes in the mangroves. DOC, dissolved organic carbon. (Adapted from Lee *et al.*, 2014.)

and *Sonneratia* are often at the seaward edge, followed by *Rhizophora* and finally *Bruguiera*, *Ceriops*, *Xylocarpus* and *Heritiera* at the back (Watson, 1928).

Mangrove ecosystems provide coastal protection, carbon sequestration, carbon sources for downstream ecosystems, trapping of sediments and nutrients, and habitat provision. They are nursery grounds for numerous marine species, including commercially important fish species. It is estimated that mangrove ecosystems support 30–80% of the global commercial fish catch (Rönnbäck, 1999). A recent meta-analysis of 23 publications (consisting of 51 studies) also found that mangroves benefitted fishery catches across the board – be it crab, fish, shellfish or prawn fishery (Carrasquilla-Henao and Juanes, 2017). The total economic value of mangroves for the ecosystem services that they provide has been estimated to range from US\$200,000 to US\$900,000/ha/year (Wells, 2006). It is important to note that while there are fish species specific to mangrove ecosystems, many are transient visitors from adjacent connected ecosystems (e.g. seagrasses and coral reefs) that utilize the unique mangrove habitat (Lee *et al.*, 2014).

Mangroves as carbon sinks and sources

As wetland forests, the anaerobic mangrove mud outgases CO_2 and CH_4 . At a global scale, mangroves are estimated to emit 26–50 Tg C/year (Rosentreter *et al.*, 2018). However, because of their high productivity, mangroves are generally able to offset most, if not all, the greenhouse gas emission through photosynthesis (Castillo *et al.*, 2017). Mangroves are estimated to store ~31% of the global blue carbon (FAO, 2010), that is the organic carbon stored, sequestered and released from coastal estuarine and marine ecosystems (McLeod *et al.*, 2011). On average, mangroves store 1023 Mg carbon/ha (Donato *et al.*, 2011). Mangrove trees have high net productivity despite the usually limited phosphate and nitrate concentrations in the ecosystem (Reef *et al.*, 2010). They achieve self-sustained productivity by altering localized soil biogeochemistry around their roots: aboveground mangrove roots translocate oxygen underground, oxidizing the surrounding soils (Nickerson and Thibodeau, 1985; Alongi *et al.*, 2004) which help to release previously unavailable phosphate and significantly increase bacterial nitrogen fixation to enrich the soil with nitrates

(Inoue *et al.*, 2011). The carbon stored per hectare of mangroves surpasses that of other tropical rainforests (Friess *et al.*, 2016). This is not only attributed to the high productivity of mangrove trees, but also to the slower rates of decomposition of organic material in the mangrove ecosystem under waterlogged conditions, which are then accumulated in the sediment. As such, mangrove sediments can contain 49–98% of the total carbon in the mangrove ecosystem (Donato *et al.*, 2011). Carbon in mangroves can also originate from allochthonous sources, such as riverine-transported terrestrial materials, phytoplankton and seagrass detritus that are imported with the tides (Kristensen *et al.*, 2008).

With the rapid mangrove deforestation, mangroves are now at risk of becoming carbon sources. Population increase and pressures on coastal resources has led to severe mangrove loss in the last 30 decades. The past decade alone saw the highest rates of mangrove losses, particularly in the tropical mangrove biodiversity hotspots in Asia (Strong and Minnemeyer, 2015). It is estimated that ~36,000 km² of mangroves have been cleared between 1980 and 2005, with the remaining mangrove ecosystems in various states of degradation (Spalding, 2010). The main drivers of mangrove loss and degradation are coastal development, conversion to aquaculture, agriculture and, to a lesser extent, timber production. Mangroves that occur upstream are particularly vulnerable as they are often the first to be cleared for aquaculture and agriculture (Polidoro *et al.*, 2010). Rice agriculture has been the leading driver for mangrove loss in Myanmar (Richards and Friess, 2016), and aquaculture was identified as the major cause for mangrove loss in South-east Asia between 2000 and 2012. Land clearing for oil palm plantations in Indonesia and Malaysia (the top two countries with the largest expanse of mangroves in South-east Asia) will, however, soon supersede all other immediate causes of mangrove loss especially since oil palm production is predicted to increase by a further 30% above the 2012 levels by 2019 (Giri *et al.*, 2011; Richards and Friess, 2016). As mangroves are cleared, the exposed and drained mangrove soil oxidizes to release carbon dioxide into the atmosphere (Lovelock *et al.*, 2011), potentially exacerbating the rate of anthropogenic climate change. When mangroves are converted to other non-forested land use, the latter might emit less greenhouse gases, but ultimately are net sources due to the lack of vegetation to reabsorb the greenhouse gases (Castillo *et al.*, 2017).

Mangroves and rising sea level

Historically, undisturbed mangrove ecosystems have largely kept pace with rising sea level either by gradually migrating inland, or by accreting land vertically (Krauss *et al.*, 2014; Sasmito *et al.*, 2016). Mangrove roots bind and retain sediments as well as mangrove leaf litter and woody debris, which will eventually decompose and add to deposits on the soil surface. Mangrove roots also influence subsurface changes: for example, high soil bulk density or low nutrients both induce root accumulation, which in turn expands soil volume. These processes allow mangrove ecosystems to alter soil elevation and keep up with sea level changes, and vertical accretion rates ranging from 0.7 mm/year to 20.8 mm/year have previously been recorded (Krauss *et al.*, 2010). The impact of mangrove roots on soil level is so significant that even scale harvesting of individual trees can reduce elevation by reducing root growth and higher root decomposition (Krauss *et al.*, 2010). Changes to surface elevation (e.g. sedimentation, groundwater influx, land movement/uplifting) combined with biological factors drive the net losses or gains in soil elevation within a mangrove area.

The predicted rise in sea level as well as increasing frequency of storm surges and extreme high-water events associated with anthropogenic climate change, would increase the inundation duration and confer added physiological stress to the mangroves (Alongi, 2008; Gilman *et al.*, 2008; Krauss *et al.*, 2014). There is a real concern that mangrove ecosystems may not be able to keep pace with the rate of rising sea levels (global average rate ~3.2 mm/year) (see Section 2.1.4), especially for slower accreting mangrove systems. Increased flooding duration is predicted to decrease root growth (Castañeda-Moya *et al.*, 2011), leading to soil subsidence and reduced surface elevation (Krauss *et al.*, 2010), which will further exacerbate the effects of rising sea level. Drought events (e.g. due to changes in rainfall patterns associated with climate change) or excessive nutrients (e.g. from agricultural discharge) can also slow down root growth (Castañeda-Moya *et al.*, 2013). Furthermore, increased coastal development also prevents mangroves from migrating inland, and hence it is predicted that mangroves will decrease into narrower belts around coastal areas.

Mangroves are predicted to keep up with medium but not the highest Intergovernmental Panel on

Climate Change (IPCC) sea level rise scenario to the end of this century (Sasmito *et al.*, 2016). Krauss *et al.* 2014 concluded from eight studies, which spanned from 1 to 6 years, that tide-dominated fringe mangroves would be the most impacted by rise in sea level, followed by overwash islands, then basin and interior mangroves. Deforested mangroves generally suffered the greatest elevation loss, while extreme subsidence also occurred at mangroves that were impacted by urban development (Krauss *et al.*, 2010). Whether or not mangrove ecosystems will keep pace with rising sea level and other climate-change-related disturbances will likely depend on species traits and adaptability, as well as interactions and adaptations at the community level. Generalized predictions are hard to make because of different and changing local hydro-geomorphological attributes, both natural and man-induced ones, which impact sediment inputs to the mangroves (Friess *et al.*, 2012; Krauss *et al.*, 2014). While increasing atmospheric carbon dioxide concentrations are generally thought to be beneficial for mangrove trees, these effects are likely to be dependent on other changing parameters, such as salinity and nutrients (Krauss *et al.*, 2014).

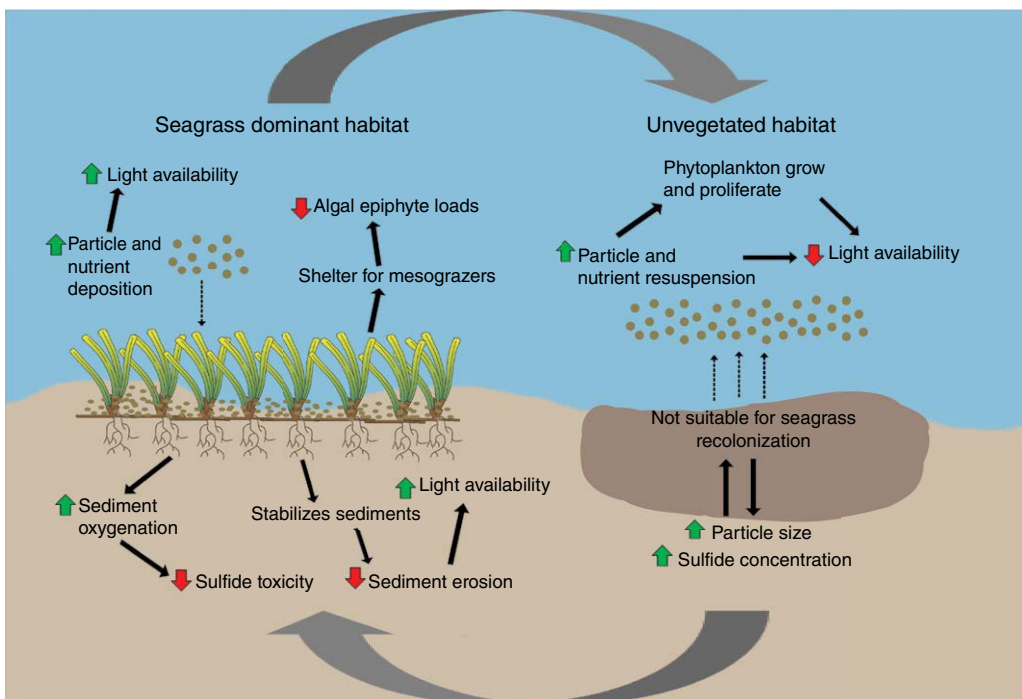
2.2.2 Seagrass ecosystems

Seagrasses are marine flowering plants that can form dense underwater meadows which form the basis for tropical seagrass ecosystems. They alter the physico-chemical properties of their environment. Seagrasses release oxygen and organic carbon to the water column and sediments during photosynthesis (Moriarty *et al.*, 1986; Brodersen *et al.*, 2015), and 'pump' nutrients from the sediments through their roots while releasing them into the water through their leaves (Romero *et al.*, 2006). Vertical extension of their leaves also reduces water flow and increases the deposition of suspended particles, while their underground rhizomes and roots stabilize the sediment and potentially reduce erosion (Christianen *et al.*, 2013). Compared to bare substrate (e.g. sand or silt), seagrasses offer a high degree of structural complexity as well as nutrition, providing shelter and food for a diverse community of organisms. Abundance, species diversity and growth rates of fish and motile invertebrates are greater in seagrass habitats than in non-vegetated habitats (e.g. Arrivillaga and Baltz, 1999; Heck *et al.*, 2003;

Bloomfield and Gillanders, 2005). The high primary productivity of seagrass meadows is also an important source of nutrients and organic material for adjacent coastal ecosystems (e.g. coral reefs, sand banks) when exported as living or dead leaves (Gillis *et al.*, 2014). Despite occupying only 0.1% of the total ocean floor, seagrasses can be responsible for up to 11% of the organic carbon buried in the ocean (Fourqurean *et al.*, 2012). Algae and microalgae (epiphytes) that grow attached to seagrass leaves are grazed on by snails and crustaceans, while the leaves are eaten directly by turtles and marine mammals (dugongs and manatees). Congregations of smaller organisms and juveniles in seagrass meadows attract larger animals (e.g. fishes and snakes) to hunt, creating a complex and diverse marine ecosystem (Valentine and Heck, 1999). Coral reef fishes, including those belonging to the families Siganidae, Lutjanidae and Mullidae (e.g. *Siganus spinus*, *Lutjanus monostigma*, *Parupeneus barberinus*), also move between seagrass and reef habitats in accordance to their life cycles and/or their feeding strategies (Grober-Dunsmore *et al.*, 2007). Owing to their importance as feeding and nursery grounds for many commercially important marine species, seagrass ecosystems have global significance to fisheries (Nordlund *et al.*, 2017).

Areal cover of seagrass meadows has been disappearing at a rate of 7% per year since 1990 (Waycott *et al.*, 2009). Most of the losses in recent decades have been attributed to increased human activities along the coast (Orth *et al.*, 2006) (Fig. 2.5). Nutrient loading, from increased land runoff and fertilizer use, can lead to eutrophication and algal blooms, outcompeting seagrasses (Kroon *et al.*, 2012). Land clearing and development in catchment areas as well as dredging (Erftemeijer and Lewis, 2006) increases sediment transport and deposition (i.e. loading) to coastal areas. Increased sediment loading can physically smother seagrass (Brodersen *et al.*, 2017) and block out sunlight (Ralph *et al.*, 2007), resulting in large-scale losses of seagrasses. Habitat fragmentation and physical damage resulting from boat traffic and coastal construction also increases erosion in seagrass habitats as well as interfering with animal movement within the meadow (Mizerek *et al.*, 2011). These mostly localized disturbances are becoming increasingly compounded by larger scale impacts of climate change, further threatening tropical seagrass ecosystems globally.

- Major loss mechanisms:
- acute heat and/or salinity stress;
 - storm events;
 - direct smothering by sediments; and
 - chronic decline in water quality (e.g. eutrophication).



- Recovery takes > 10 years. Subject to:
- repeated disturbances (e.g. storms, sediment discharge);
 - water quality management; and
 - restoration efforts.

Fig. 2.5. In seagrass-dominant habitats, seagrasses ‘engineer’ their environment via feedback mechanisms. After a devastating loss in seagrass, the ecosystem flips to an alternate regime where the absence of vegetation hinders seagrass recolonization via feedback. Green arrows indicate an increase and red arrows indicate a decrease in levels. Ecosystem services associated with meadow productivity and structural complexity, such as provision of food and shelter for finfish and shellfish, are attenuated or lost. Recovery typically takes a long time and is subjected to many factors. (Adapted from Maxwell *et al.*, 2017.)

Seagrasses and rising temperatures

Waters within the tropical Indo-Pacific region, which hosts around one-fifth of the world’s 72 seagrass species, are predicted to warm up faster than global average rates (Waycott *et al.*, 2004; Lough, 2012; Figs 2.1 and 2.2). With rising water temperatures, increased respiratory carbon loss reduces net productivity in seagrasses. In the tropics, some species live near to their thermal limits and will have to upregulate stress-response systems to withstand exposure to sublethal temperatures

with climate change (Koch *et al.*, 2013). Under chronic temperature stress, seagrasses reduce their growth, plant size and the number of shoots and leaves (Collier *et al.*, 2011; Thomson *et al.*, 2015). Lowered primary productivity and a thinned-out seagrass canopy attenuate the ecological benefits provided to its denizens, and these effects are likely to cascade through the trophic system. Short-term thermal events called ‘heat waves’ are predicted to increase in frequency and intensity due to climate change (Solomon *et al.*, 2007). Marine heat waves have the devastating potential to abruptly decimate

seagrass populations and impact the health of associated organisms such as turtles (Thomson *et al.*, 2015) and fish (Wernberg *et al.*, 2013). A drastic loss and shift in biomass and structural complexity in the meadow (Nowicki *et al.*, 2017) was observed after the 2011 heat wave along the Western Australian coastline.

Seagrasses and ocean acidification

With ocean acidification, higher levels of dissolved inorganic carbon (DIC) will be available for photosynthesis (Koch *et al.*, 2013). Tropical seagrasses can upregulate photosynthetic rates with DIC enrichment (Jiang *et al.*, 2010; Russell *et al.*, 2013; Ow *et al.*, 2015) and possibly increase biomass (Palacios and Zimmerman, 2007). At shallow submarine carbon dioxide vents where seagrasses were exposed to long-term DIC enrichment, seagrass cover and biomass were three- to fivefold greater than at non-vent sites (Takahashi *et al.*, 2015). This shows that seagrasses can potentially thrive as the ocean becomes more acidic with anthropogenic climate change. However, not all species will present plant-scale responses in the same way, due to interspecific variation in growth strategies (Hemminga and Duarte, 2000) and DIC utilization (e.g. Campbell and Fourqurean, 2013; Ow *et al.*, 2016a). A shift in seagrass community composition and structure within tropical multispecific meadows is expected (Takahashi *et al.*, 2015). A shift in the epiphytic community – a reduction in calcareous organisms and increased colonization of non-calcifying algae (Campbell and Fourqurean, 2014) – will potentially affect grazer communities by altering food availability in seagrass meadows.

Seagrasses and rising sea level

Sea level rise will increase water depth which will attenuate light penetration to seagrasses. Seagrasses that are distributed at their lowest depth limits (i.e. at minimum light requirement), such as those which are already affected by declining water quality due to nutrient or sediment inputs from coastal development and dredging, could be particularly vulnerable to sea level rise (Waycott *et al.*, 2009). To cope with sea level rise, seagrasses will have to adapt or acclimatize to the new light and hydrodynamic conditions, and accretionary processes in the habitat will have to keep up with rising sea level (Short and Neckles, 1999; Duarte, 2002). Furthermore, as

new regions become inundated, seagrass could potentially migrate into newly available areas (Duarte, 2002). However, in highly developed urban areas, this inland migration will be prevented by armoured shorelines (Nicholls, 2011). So far, research on the effects of rise in sea level on seagrass have been limited to modelling studies developed for temperate seagrass habitats in North America (Kairis and Rybczyk, 2010; Shaughnessy *et al.*, 2012) and subtropical habitats in Australia (Saunders *et al.*, 2013). These studies found that predicted changes in seagrass cover were variable (Kairis and Rybczyk, 2010; Shaughnessy *et al.*, 2012; Saunders *et al.*, 2013). Empirical evidence will be needed to further determine the impacts sea level has on seagrass ecosystems.

Seagrasses and changes in rainfall and increased storm activity

Storm events can cause large-scale decimation of seagrass meadows. Storm surges stir up large quantities of sediments, which can lead to further die-off due to burial, smothering and increased turbidity (Ralph *et al.*, 2007; Brodersen *et al.*, 2017). Increased rainfall amounts are also predicted to deliver higher sediment and nutrient loads from terrestrial runoff and river discharges into the coastal system (e.g. Justic *et al.*, 2005). Acute turbidity events have been linked to seagrass loss through a sharp decline in photosynthetic rates and shoot densities (Ralph *et al.*, 2007; Petus *et al.*, 2014). This will further compound the stress experienced by seagrasses during storm events, and hamper their ability to recover (Rasheed *et al.*, 2014). As storm events are predicted to increase in frequency and intensity, seagrass ecosystems will likely have narrower recovery windows between disturbances.

Sheltered coastal seagrass meadows may be especially vulnerable to changes in rainfall patterns. Reduced rainfall results in low coastal freshwater discharge into sheltered bays leading to an increase in salinity. While tropical seagrasses are tolerant of high salinity (Koch *et al.*, 2007a), prolonged exposure combined with high temperatures and low circulation simultaneously enhance the metabolic demands of the plants and reduce the amount of oxygen that remain dissolved in seawater (Koch *et al.*, 2007b). Anoxic conditions drive bacteria in the water column and sediments to produce hydrogen sulfide, a known phytotoxin, during respiration

(Borum *et al.*, 2005). As seagrass dies and decays, more dissolved oxygen is consumed and more hydrogen sulfide is produced – fuelling further seagrass mortality. In 2015, a 3-month delay in seasonal summer rainfall over Florida Bay preceded a massive die-off of seagrass. A staggering 22,000 acres of habitat lost their seagrass cover and localized fish kills were observed in the same areas (Hall *et al.*, 2016).

Interactions between climatic changes and other anthropogenic influences

Whether increases in atmospheric carbon dioxide projected for the next century will be able to sufficiently stimulate photosynthesis in seagrasses to offset the negative effects of thermal stress remains largely unknown for tropical seagrass ecosystems. Thus far, experimental work revealed limited effects of DIC enrichment across a range of temperatures (21–35°C) in tropical seagrasses (Collier *et al.*, 2018). Turbidity, due to increased sedimentation, can aggravate the negative effects of warming as seagrasses growing at higher temperatures tend to have higher light requirements (Collier *et al.*, 2011; Nowicki *et al.*, 2017). An increase in nitrogen load (e.g. fertilizers, sewage) on coastal ecosystems could theoretically augment DIC responses (Stitt and Krapp, 1999). However, nutrient increases (from 0.3 μm to $\sim 2\mu\text{m}$ dissolved inorganic nitrogen, approximating to Great Barrier Reef flood plume levels) had negligible effects on seagrass growth in enriched DIC (Ow *et al.*, 2016b). Conversely, seagrass epiphytes and macroalgae are predicted to greatly benefit from nutrient and DIC enrichment. Competition from epiphytes and algae are likely to negate positive ocean acidification effects on seagrass growth (Burnell *et al.*, 2014).

2.2.3 Coral reefs

Coral reefs occupy < 0.1% of the world's oceans but they account for and support > 25% of the biodiversity in these oceans. While coral reefs flourish within the Tropic of Cancer and Tropic of Capricorn, the most diverse coral reef communities can be found in the 'Coral Triangle'. The high species diversity (estimated to be 1–9 million species) and gross productivity on coral reefs generate goods and services that directly or indirectly support hundreds of millions of people worldwide (Spalding and Grenfell, 1997; Mulhall, 2007).

In developing countries, approximately 6 million t of fish, or $\sim 25\%$ of total fish catch, are caught from the tropical reefs each year (Jameson *et al.*, 1995). The calcium carbonate framework of coral reefs also provides significant coastline protection, especially important for small islands, coastal wetlands, ports and harbours, by absorbing wave energy and mitigating shoreline erosion (Wilkinson and Buddemeier, 1994; Grimsditch and Salm, 2006). Coral reefs support billion-dollar industries such as fisheries and tourism, and are hot spots for the pharmaceutical industry. Dozens of current and potential antimicrobial and anti-inflammatory medications have been discovered from reef organisms (Birkeland, 1997; Mulhall, 2007). In the world economy, it is estimated that coral reefs provide about US\$30 billion of net benefits in goods and services.

'Reef-building' hermatypic scleractinian corals form the backbone of the ecosystem. These animals, which live in symbiosis with algae called zooxanthellae (*Symbiodinium* spp.), have adapted to living optimally in a relatively narrow temperature window of $\sim 23\text{--}29^\circ\text{C}$ (Kleypas *et al.*, 1999; Guan *et al.*, 2015). As they grow, corals produce large amounts of CaCO_3 skeletons ($\sim 2\text{--}6\text{ kg/m}^2/\text{year}$) (Barnes and Devereux, 1984) to construct massive three-dimensional structures that ultimately form the underlying framework for life on tropical coral reefs (Bellwood and Hughes, 2001; Chabanet *et al.*, 2005). The high productivity and biodiversity associated with coral reefs are therefore dependent on the very production and maintenance of this CaCO_3 framework by corals as key ecosystem engineers, set against a background of biogeochemical erosion and physical damage caused by humans, storms, grazers and bioeroders to which they are exposed (e.g. Chen *et al.*, 2012; Ferrari *et al.*, 2012; Meko *et al.*, 2012).

There is rising concern that the rates of changes in climate conditions could exceed the adaptive capacity of tropical coral reefs worldwide beyond the point of no return. Detrimental effects of reduced water quality associated with regional/local-scale coastal development now overlap with global-scale impacts of climate change, and short-term pulse events (e.g. anomalous spikes in sea temperatures linked to disturbances to ENSO) also coincide with longer-term chronic changes (e.g. eutrophication and ocean acidification; Fig. 2.6) (Nyström and Folke, 2001; Chabanet *et al.*, 2005). This increasing exposure to multiple, concurrent

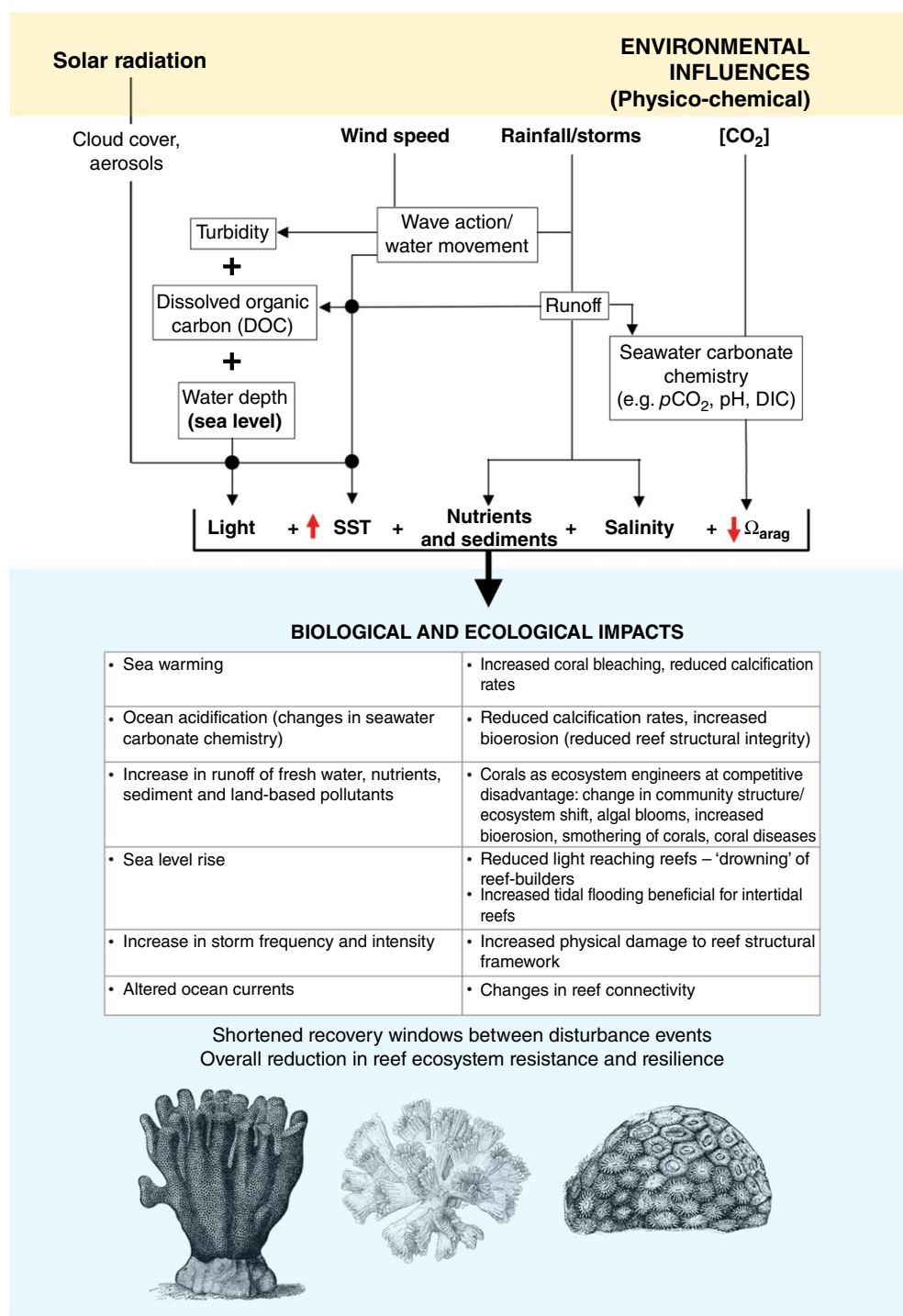


Fig. 2.6. A summary of environmental influences, their interactions and the predicted biological and ecological impacts on coral reefs. DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; Ω_{arag} , aragonite saturation state; pCO₂, partial pressure of carbon dioxide; SST, sea surface temperature.

stressors severely compromises reef health and resilience, consequentially upsetting the balance between disturbance and reef recovery and causing their decline worldwide (Wilkinson, 1996; Moberg and Folke, 1999; Folke *et al.*, 2004; Heron *et al.*, 2016; Hughes *et al.*, 2017a, b). Major factors associated with global climate change predicted to negatively impact coral reefs globally are rising ocean temperatures and ocean acidification (Gattuso *et al.*, 1998; Kleypas *et al.*, 1999; Marubini and Atkinson, 1999; Ohde and van Woesik, 1999; Langdon *et al.*, 2000, 2003; Leclercq *et al.*, 2002; Marubini *et al.*, 2003; Orr *et al.*, 2005; Pandolfi *et al.*, 2011; Tanzil *et al.*, 2013; Heron *et al.*, 2016; Hoegh-Guldberg *et al.*, 2017; Hughes *et al.*, 2017a, b). Other climate-change-related factors not to be overlooked that could also potentially affect tropical reefs include: (i) rising sea level (Brown, 1997; Hubbard, 1997; Hoegh-Guldberg, 1999; Anderson *et al.*, 2010; Brown *et al.*, 2011); (ii) changes in rainfall patterns (Fabricius, 2004; Justic *et al.*, 2005; Jones and Berkelmans, 2014); and (iii) frequency and intensity of storm events (Woodley *et al.*, 1981; Harmelin-Vivien and Laboute, 1986; Scoffin, 1993; Heron *et al.*, 2005; Fabricius *et al.*, 2008).

Coral reefs and sea warming

Rising ocean temperatures associated with anthropogenic climate change is of particular concern pushing tropical reef-building corals beyond the upper limits of their thermal tolerance (Cooper *et al.*, 2007; Tanzil *et al.*, 2013; Heron *et al.*, 2016; Hughes *et al.*, 2017a). Temperature stress can disrupt symbiosis resulting in the death or expulsion of the coral's zooxanthellae – a phenomenon known as coral bleaching and visibly seen as a loss of colouration (Douglas, 2003). Physiologically, this loss of photo-endosymbionts translates to the decline of a major energy resource for the coral host (Muscantine *et al.*, 1981; Davies, 1984; Grottoli *et al.*, 2006), and processes such as calcification can all but cease during this stressful period (Goreau and MacFarlane, 1990; Tudhope *et al.*, 1992). Thermal bleaching in other associated reef organisms, such as anemones and soft corals, have also been observed (e.g. Chavanich *et al.*, 2009; Tun *et al.*, 2010; Hobbs *et al.*, 2013). Since the 1970s, reports of mass coral bleaching have increased in frequency, with severe bleaching events coinciding with anomalous sea warming

events related to the ENSO (Hoegh-Guldberg, 1999; Oliver *et al.*, 2009; Eakin *et al.*, 2009; Hughes *et al.*, 2017b). The most severe pan-tropical coral bleaching episodes were observed in 1997–1998, 2010 and 2015–2016 – triggered when sea temperatures rose to 2°C above the seasonal maxima (Hoegh-Guldberg, 1999; Tun *et al.*, 2010; Heron *et al.*, 2016; Hughes *et al.*, 2017b). These events resulted in many reefs losing up to 90% of their live coral cover (Tun *et al.*, 2010; Phongsuwan and Chansang, 2012; Hughes *et al.*, 2017a). Recovery following such bleaching and mortality events is possible, and they vary from 2 years to > 10 years. Recovery periods are dependent on coral growth rates, colonization rates (i.e. rates of reproduction, settlement, recruitment), water quality, and fish populations (in particular presence of algal grazers) (Suefuji and van Woesik, 2001; Kayanne *et al.*, 2002; Phongsuwan and Chansang, 2012; Gilmour *et al.*, 2013; Pisapia *et al.*, 2016; Hughes *et al.*, 2017b). As average global temperatures continue to rise, mass bleaching events are predicted to increase both in intensity and in frequency, further reducing the intervals between disturbances as well as time windows for reef ecosystem recovery.

Coral reefs and changing seawater carbonate chemistry

Decreases in seawater calcium carbonate saturation state (Ω) related to acidifying oceans are predicted to significantly reduce the rates of calcification (i.e. production of biogenic CaCO_3) by calcifying reef organisms (Gattuso *et al.*, 1998; Kleypas *et al.*, 1999; Kleypas and Langdon, 2006; Silverman *et al.*, 2007, 2009; Friedrich *et al.*, 2012). These include corals, but also calcareous algae (e.g. crustose coralline algae), tubeworms (e.g. serpulids) and foraminifera (Yamano *et al.*, 2000; Jokiel *et al.*, 2008; Comeau *et al.*, 2015). For reef-building corals, results from laboratory experiments show that for every unit decrease in Ω (of aragonite), average calcification rates reduced by ~5–40% (Langdon and Atkinson, 2005; Kleypas and Langdon, 2006) – variability in these rates is likely in part due to varying responses by different coral species, if not differences in experimental setups. Based on extrapolations, reef coral calcification is estimated to have declined by 6–15% over the past century, and predicted to further decrease by 17–60% relative to pre-industrial levels

by the end of the 21st century (Kleypas *et al.*, 1999; Silverman *et al.*, 2007, 2009; Friedrich *et al.*, 2012). Negative impacts of ocean acidification are also expected to be more pronounced for corals living in warmer equatorial waters than in higher-latitude relatively cooler waters (Reynaud *et al.*, 2003; Anthony *et al.*, 2008; Prada *et al.*, 2017). For example, Reynaud *et al.* (2003) reported higher reductions in calcification rates in corals subject to increased partial pressure of carbon dioxide ($p\text{CO}_2$) at higher temperatures ($\sim 28^\circ\text{C}$) than at lower temperatures ($\sim 25^\circ\text{C}$). Surveys of coral reefs naturally exposed to elevated seawater $p\text{CO}_2$ (pH 7.73–8.00; aragonite saturation state (Ω_{arag}) 2.9) also found lowered coral diversity, coral recruitment, bioerosion, reef framework complexity and shifts in the reef community structure compared with adjacent ‘control’ sites (pH 7.97–8.14; Ω_{arag} 3.5) (Fabricius *et al.*, 2011). Massive *Porites* corals and non-calcareous macroalgae dominated the high $p\text{CO}_2$ sites, indicating changes in competitive interactions between taxa. In addition to lowered calcification rates, reefs may also face increased rates of net dissolution through chemical as well as biological erosion rates at low pH, further risking the integrity of the reef carbonate framework (Wissihak *et al.*, 2012; Crook *et al.*, 2013). Any loss of complexity in the three-dimensional framework can further impact a large variety of marine organisms within the reef ecosystem by reducing habitat complexity and the availability of refuges (Fabricius *et al.*, 2014).

Coral reefs and rising sea level

Rising sea levels are predicted to affect the amount of light that shallow coral reefs currently receive (Hoegh-Guldberg, 1999; Field *et al.*, 2011). Currently, sea level rise is occurring more slowly than coral growth rates and therefore may have limited or even a positive impact on coral reefs, particularly for intertidal reefs (Brown, 1997; Hubbard, 1997). However, the accumulation of other environmental disturbance which can slow growth rates may expose corals to the effects of rising sea levels, possibly ‘drowning’ them when they are unable to keep up with rises in sea levels. For coastal coral reefs, sea level rise can also translate into increased coastal erosion and advection of fine sediment to adjacent reefs and increasing seawater turbidity and sedimentation rates – known stressors of coral reefs (Fabricius, 2004).

Coral reefs and changes in rainfall and increased storm activity

Predictions of changing rainfall patterns and higher intensity rain/storm events over the coming decades, combined with the anthropogenic influences on water quality, are likely to slow reef regeneration and impact on long-term reef resilience. Storms affect the hydraulic energy and physical damage received by reefs as well as increases in terrestrial runoff and riverine inputs into the coastal system (e.g. Fabricius, 2004; Justic *et al.*, 2005). Storm events have been known to cause physical destruction of the reef structure and organisms by increased wave action and subsequent movement of coral rubble, and increased sedimentation and turbidity (through resuspension and runoff) which can hinder reef recovery processes (Brown, 1997; Gardner *et al.*, 2005). During such events fragile coral growth forms (i.e. branching and foliose) suffer greatly from breakage and more massive forms from dislodgement, which can cause them to fall down steep slopes and possibly cause further damage (Woodley *et al.*, 1981; Harmelin-Vivien and Laboute, 1986; Heron *et al.*, 2005).

Nearshore reef systems are likely to be most impacted by any increased terrestrial runoff and riverine inputs into the coastal environment. Increased sediment loading on already turbid reef environments will further reduce photosynthetically active radiation (PAR) reaching reefs (Dunne and Brown, 1996; Fabricius, 2004; Justic *et al.*, 2005). Increased turbidity and reduction in PAR decreases the photosynthetic abilities of reef autotrophs and increases respiration rates, thereby reducing the ratio between photosynthesis and respiration (Anthony and Fabricius, 2000; Anthony and Connolly, 2004). In extremely turbid reef environments, such as those found around Singapore, it has been shown that corals at a water depth of ~ 3 m are barely meeting their daily energy needs solely from photosynthesis (Tun *et al.*, 1994). Additional reductions in light due to increased cloud cover, rising sea level and increased coastal activities (e.g. coastal developments, dredging, boat activities) could further push corals already living in such marginal conditions to their physiological limits for survival. It should, however, be noted that while the effects of increased rainfall/storm events and sea level described above are mostly negative and precautionary, these environmental disturbances might also offer ecological benefits. Increased water movement and

cloud cover could help alleviate thermal stress on corals, cooling waters through shading the sea surface from solar radiation and transfer of latent heat (evaporative cooling), reducing the impacts of rising sea temperatures (Heron *et al.*, 2005).

2.3 Future Outlook for Important Tropical Marine and Brackish Ecosystems

2.3.1 Future changes in ocean, coastal and estuarine environments

In general, tropical oceans will continue to become warmer, less saline, more productive and acidic as climate change progresses, exacerbated by greenhouse gas emissions. Interactions of changing abiotic variables are also likely to result in complex outcomes. Changes in ocean circulation patterns and an anticipated increase in upwelling of deep ocean waters will bring more acidic and enriched water to the upper ocean layers. Meanwhile, atmospheric increases in temperature and carbon dioxide will warm and acidify the upper layers of the ocean. Wetter areas with higher precipitation and greater runoffs from fresh waters become 'more fresh' while localities with less precipitation will become more salty. Warmer, more enriched and acidic waters driven by oceanic processes, along with changes in the quantity and quality of freshwater inputs will probably result in increased eutrophication of coastal and estuarine waters (Sinha *et al.*, 2017). Warmer waters will work in concert with elevated nutrient loads to increase production. In turn, the synergistic effects of temperature and deoxygenation of seawater will be likely to exacerbate the problem of eutrophication in coastal waters, as excessive algal biomass decomposes via bacterial action and oxygen is further consumed from the water column.

In dynamic coastal and estuarine environments, which form the interface between land and sea and are less influenced by open-ocean forcing, large variability in responses are expected as local conditions continue to interact with and mediate global effects. It is also likely that different coastal regions will be impacted differently by different stressors (e.g. salinity, sea level or ocean acidification). In such coastal areas, freshwater runoffs from terrestrial environments to the sea will be a major factor influencing both the quantity and the quality of receiving waters. In the absence of human influence, volumes of runoffs would reflect precipitation

patterns whereby wetter areas receive greater rainfall reflected in runoffs to the sea, with dry areas becoming drier. Given the increasing global demand for water resources for use and storage by a growing population, reductions in the volumes of fresh water reaching the sea are expected to continue, if not managed differently, while loads of pollutants including nutrients may well increase.

2.3.2 Implications for marine and brackish ecosystems

The future looks generally bleak for important tropical marine and brackish ecosystems as climate change impacts continue to compound with other human-related local/regional-scale disturbances. A recurrent theme within this chapter is that we are already seeing major and fundamental changes in the marine environment in response to climate change, and that the rate of change is largely outstripping the ability for important yet sensitive ecosystems, such as coral reefs, seagrasses and mangroves, to adapt genetically or relocate. There are, however, rays of hope that tropical marine and brackish ecosystems face in an uncertain future. In coastal and marine systems there is evidence of 'resistance' and 'resilience' (i.e. the capacity of systems to withstand or tolerate stress – 'resistance'; and to adapt and self-repair/recover from disturbances – 'resilience') so as to still retain essentially the same functions (Walker *et al.*, 2004; Dizon and Yap, 2006). For example, there are reefs in the Gulf of Aqaba (GoA), Red Sea, that have remained bleaching-free in the last three decades, despite experiencing anomalous SSTs exceeding local average summer maximum by as much as 2°C. Some corals from the GoA are able to withstand high thermal stress of up to 33°C, 6°C warmer than the average summer SST (Fine *et al.*, 2013). There are also reefs that exist in highly urbanized environments with elevated sedimentation, suspended sediments, nutrients, turbidity, depressed light penetration and fluctuating salinities, such as those in Singapore and along the Malacca Straits (Tun, 2012; Tanzil *et al.*, 2013). Such examples seem to defy prevailing conceptual paradigms of ecosystem disturbance and show heterogeneity in ecosystem responses and highlight the potential for some areas to become ecosystem refuges that allow for acclimatization and/or adaptation.

To mitigate the effects of climate change on ecosystems, active conservation and restoration measures

are also necessary. Rehabilitation and restoration efforts, while costly (Bayraktarov *et al.*, 2016), help ecosystem recovery. For example, growing roots of reforested mangroves helped to re-establish subsurface elevation (McKee, 2011). After 20 years, transplanted mangroves in the Philippines have regained about half of the mangrove specialist fish species, and more for the non-specialist species (Honda *et al.*, 2013). Another study found that planted mangroves in India significantly increased the catch of mangrove-dependent fishes (representing both commercial and artisanal catch data), and estimated that the contribution of young planted stands increases catch by about a fourth of the natural stands (Das, 2017). Restoration efforts accompanied by water quality management through monitoring and load restrictions (e.g. of sediment and nutrients) from various sources can provide some buffer for climatic disturbances by helping to re-establish habitats and ecosystem services, and to preserve genetic diversity (Collier *et al.*, 2011).

The future fate of tropical marine and brackish ecosystems will not only depend on our ability to stabilize planetary temperature and CO₂ concentrations (e.g. by reducing or at least limiting emissions at current levels, and sequestering atmospheric CO₂) as quickly as possible, but also on local policies in managing and reducing the pressure from rising human populations on resources. By reducing non-climate stresses, ecosystems will have the opportunity to develop resilience (through acclimatization or adaptation) to the challenges of our changing planet (Hoegh-Guldberg *et al.*, 2017). Integrated environmental monitoring and management strategies that consider both upstream and downstream effects, as well as interdependencies between connected ecosystems, will likely provide the highest buffering capacities. Lastly, to promote sustainable environmental practices, scientists must accurately and effectively communicate their findings in a way that policy makers and laypersons can understand. Science communication practices that are not entrenched in the culture of evaluating 'impacts' will likely become more socially relevant and crucial for creating real-world solutions.

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3

Skeletal Abnormalities

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3.1 Introduction

The teleost skeleton is a plastic (viz. malleable) organ system whose anatomy, histology, mechanical properties and number of elements is influenced by environmental as well as genetic factors. The skeleton has many functions which include mechanical (protection, locomotion, feeding), signalling (i.e. notochord during the ontogenesis), regulatory (mineral metabolism; production of several hormones), storage (i.e. lipid, phosphorus), communication (sound production and reception) and generation of secondary sexual characters (Witten and Hall, 2015). This complex relationship between the skeleton and environmental, genetic and epigenetic factors, on the one hand, and ecology, biology and physiology of fish on the other, has attracted much attention (e.g. from palaeontologists, evolutionists, developmental biologists, ecologists, biomechanists, geneticists, physiologists, immunologists and anatomists).

The fish skeleton includes a plethora of different types of bone and cartilage and also many skeletal tissues between connective tissue and bone, and bone and cartilage. This is in contrast to terrestrial vertebrates. Each of these skeletal tissues undergoes metabolism-related processes as differentiation, trans-differentiation, modelling and remodelling along the entire life cycle of the fish. Many teleost species never stop growing; therefore, all these processes continue throughout life. Furthermore, they can repair fractures and some anomalies, and regenerate some dermal skeletal elements such as teeth, scales and fin rays (Boglione *et al.*, 2013). Consequently, several changes may simultaneously affect the skeleton of one individual fish during its life cycle (Witten and Hall, 2015).

Skeletal abnormalities or anomalies are differences or deviations from the average shape or number of skeletal elements. In this chapter, I will analyse in the context of potential climate effects:

- *altered meristic counts* – This is the variation in the number of the species-specific countable structures partly heritable and partly dependent on a range of environmental factors during development, affecting developmental rates (i.e. fin spines and rays, gill rakers, lateral line scales and branchiostegal rays). This category also includes fluctuant asymmetry that is the random deviation of a character from perfect bilateral symmetry.
- *deformations* (i.e. alteration in shape of previously normally formed elements) – including malformations (primary structural defects resulting from a localized error of morphogenesis), syndromes (sets of abnormalities occurring together) and developmental abnormalities connected to the toxic effects of teratogens in the environment.

3.2 Meristic Counts

During ontogenesis, the expression of a genetically predetermined phenotype is modulated by homeostatic control of morphological development, or developmental stability, which acts against environmental and genetic disturbances through canalization (*sensu* Waddington, 1942) or by reducing the phenotypic variation associated with a particular trait (Allenbach, 2011). Developmental stability is the capacity of a genotype to express constantly and precisely the same phenotype when exposed to the same environmental conditions during development. However, when extensive environmental

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and/or genetic stress prevails over homeostasis, different homeorhetic trajectories are established and aberrant phenotypes are produced, including altered meristic counts (Allenbach, 2011).

The assumption that individuals of a population live under specific environmental conditions makes the phenotypic plasticity useful for discriminating between marine fish populations (Berg *et al.*, 2018). Because external environmental conditions greatly influence their development, fish are often used as sentinel species for monitoring aquatic environmental health.

A substantial genetic component determines the number of vertebrae in any individual fish; however, some environmentally driven variations had been identified in the early 19th century. Latitude is the environmental factor first identified as influencing fish shape. According to Bergmann (1847) body size increases with decreasing temperature (*Bergmann's rule*). Günther (1862 in Labridae) and Gill (1864, 1865 in Acanthopterygian fishes and in *Sebastes*) acknowledged that the number of vertebrae in fishes increases with latitude. Jordan (1891), in the enunciation of the '*Law of Vertebrae*' (also called *Jordan's rule*), identified in water temperature the main driving factor for variation in the number of vertebrae in species populating different latitudes: cold-water representatives have a larger number of vertebrae than those inhabitants in tropical regions. This evolutive process, the '*ichthyization*' (Jordan, 1891), considered the reduced vertebral number evolved in warm-water species as a derived phenotype from higher vertebrae number, a consequence of skeletal specialization driven by enhanced competition. Accordingly, Hubbs (1922) identified in the different rate of temperature-mediated early ontogenetic processes the cause of the intraspecific variation in vertebral number. Lindsey in 1975, embracing *Dogiel's law of oligomerization*, postulated a reduction in vertebral number with phyletic advancement: compared with basal euteleosts, advanced perciform fishes tend to have fewer vertebrae. During a series of experiments carried out to obtain better information on the influence of the environment on the number of meristic characters in different fish species, Tåning (1952) realized that Jordan's rule is not applicable to all fish species and that temperature-driven change in vertebral number also occurred among individuals belonging to the same species. The number of vertebrae followed a U-shaped curve in sea trout, *Salmo trutta trutta* from the

same breeders but reared at different temperatures (the temperature ranges tested in different pairs of sea trout was 2.5–14°C), with the lowest average number of vertebrae at an intermediate temperature (6°C), while both higher and lower temperatures produced higher averages. The same pattern was described by Lindsey (1954) on *Macropodus opercularis* (Osphronemidae), but with an inversed U-shaped curve, while inversely linear or other shapes were highlighted by Fowler (1970) in different species. Lindsey (1962) depicted in a freshwater population of *Gasterosteus aculeatus* that total vertebral number was influenced by temperature according to a V-shaped reaction norm, with lower values located between 18 and 24°C, depending on the genotype.

In a series of experiments, Tåning (1946, 1950, 1952) demonstrated the existence of short 'super-sensitive' periods that in sea trout occurs at gastrulation shortly before the 'eyed-egg' stage when the embryo is extremely sensitive to temperature changes. This results in considerable variation in the number of vertebrae which depends on the modality of the applied temperature change. A relatively moderate shift of temperature (3–6°C) produced an average difference of about 1½ vertebrae, while abrupt changes of temperature (10–14°C; shock treatment) produced considerable changes (difference of 3–4 vertebrae), in sea trout from the same parents.

Tåning (1952) highlighted that the number of dorsal, anal and pectoral rays also reflected different changes with temperature, that they were larger and more influenced by other factors (e.g. salinity) than in the case of vertebrae, and that each fin had its own special phenotypical period in the sea trout. Hubbs (1922) and Tåning (1952) explained this as the consequence of differences in the ontogenetic phase of determination (vertebrae at embryo stage, fin rays later), these defining the time span in which environmental factors can influence development.

When Lindsey (1975) coined the concept of *pleomerism* to indicate a phenomenon in which larger individuals have more vertebrae, then latitude proved to be more strongly associated with standard length than with vertebral number. Latitude is considered a proxy for temperature, with a presumed linear relationship. Consequently, Lindsey's *pleomerism* and Bergmann's rule proved more suitable for describing the ecogeographical rules on vertebral numbers (latitude) than Jordan's rule (temperature), which was dismissed by some workers (McDowall, 2008; Morris *et al.*, 2017). However,

Jordan's rule is still a focus of research and debate (Morris *et al.*, 2017): in many species, vertebrae number increases linearly with latitude; in others the temperature proves to be associated with number of vertebrae.

Murray and Beacham (1989) acknowledged that factors influencing variation in meristic counts are complicated by genetic–environmental interactions difficult to predict. The existence of some selective pressure acting on phylogenetic lowering of number of vertebrae (Dogiel's law of oligomerization) in advanced fish is based on the tendency of fish taxa with higher vertebral counts to have higher species' and population's ranges and greater responsiveness in those counts to environmental influences (McDowall, 2004). The vertebral number, in turn, was recognized to influence locomotor performance, at least in post-larvae, and body flexibility (Jordan, 1891). In *Mylocheilus caurinus* and in *G. aculeatus*, a more direct relation with burst swimming performance of larvae was observed in the ratio of abdominal to caudal vertebrae (Swain, 1988, 1992). Other environmental factors have some phenotypical influence on vertebrae numbers and they include oxygen tension, light intensity (MacCrimmon and Kwain, 1969), regime (Tibblin *et al.*, 2016), the 'space factor' (fish which live in large, extensive areas have a higher average number of vertebrae than those in small, limited areas) (Vladykov, 1934), salinity (more correlated with the number of rays but not with vertebrae) and carbon dioxide (Tåning, 1952).

Examples of the complexity of eco-geographical-physical (and eco-evo-devo) interactions are particularly evident in a widely distributed species, such as *G. aculeatus*. Recent investigations carried out on the variation in the vertebral number in *G. aculeatus* populations differing for morphological and ecological (anadromous, benthic and limnetic) traits (Aguirre *et al.*, 2014), and in marine populations dispersed along a 21.8° latitudinal cline (Morris *et al.*, 2017) highlighted that:

- Variation in vertebral number was associated with body shape variation (a variation from Lindsey's pleomerism), but external similarity in body form masks significant vertebral variation traits (Aguirre *et al.*, 2014). Further, pleomerism was verified, especially in the caudal region, but this varied according ecological traits (benthics versus limnetics; anadromous versus either) and among populations (Aguirre *et al.*, 2014).

- Jordan's rule was confirmed, but vertebral number variation exhibited an abrupt transition (southern populations with similar lower numbers; northern with similar higher numbers) than a smooth linear gradient (Morris *et al.*, 2017). This contradicts a previous study carried out on the same species on the opposite (east Canadian) coast by Garside and Hamor (1973) along a smaller cline (less than 6° latitude) and which reported no evidence for Jordan's rule. Furthermore, if temperature is the driver of Jordan's rule, the prediction of finding similar variations in fish of the same species experiencing similar temperatures (i.e. Lindsey, 1962) should have held true: in reality, the degree of variation shown according to the latitude for each meristic trait was always very low (< 10%) (Morris *et al.*, 2017).
- Vertebral homeosis (transformation of vertebral identities) was common (individuals with more caudal vertebrae have fewer abdominal vertebrae and vice versa) and present both in differing (Morris *et al.*, 2017) and not-differing total vertebrae (Aguirre *et al.*, 2014). It proved to be associated with sexual dimorphism (males with more caudal vertebrae than females, females with more abdominal vertebrae than males) (Morris *et al.*, 2017). The synthesis could be that Jordan's rule occurred due to an increase in caudal vertebral number with increasing latitude in males, but an increase in total vertebral number in females (Morris *et al.*, 2017). The adaptive reasons for shift in vertebral count according to latitude could be linked to functional consequences for flexibility, maximum body curvature for escape swimming, body elongation associated with ambush predation, burst swimming and C-start velocity (Morris *et al.*, 2017). Furthermore, a higher ratio of caudal to abdominal vertebrae is important for predator avoidance (Swain, 1992), while a lower ratio may be necessary for the expansion of the abdominal cavity associated with egg production (Aguirre *et al.*, 2014).
- Bergmann's rule was validated as the standard length increased with increasing latitude more strongly than with vertebral number (Morris *et al.*, 2017). It has been proposed that Bergmann's rule is due to increased longevity caused by a slower growth rate and reduced competition (Angilletta and Dunham, 2003; Rypel, 2014). Moreover, taking into consideration migratory

behaviour of marine *G. aculeatus* (including juveniles) over large distances (Cowen *et al.*, 1991), both Bergmann's rule and Jordan's rule could be adaptive, particularly for the more viscous waters of the north (Herbing, 2002; McDowall, 2003; Aguirre *et al.*, 2014).

- The number of anal and dorsal fin rays and pterygiophores increases with increasing latitudes; the pectoral rays show an opposite trend (Lindsey, 1962).

It appears that all the different evolutive, genetic, geographical and physical rules apply in the determination of vertebral counts: the count in a species may be responsive to environmental differences, typically in fish with higher vertebral counts, and both vertebral number and range of variation change along with the phyletic advancement. On the other hand, the vertebral number changes with the size, shape and swimming mode of the fish, and body shape changes according to swimming modality optimized for food search and to counteract water viscosity, according to the developmental stage, ecological niche and autoecology (species-specific adaptations to environmental condition, *sensu* Walter and Hengeveld, 2000). Another intriguing connection is that evolutionary advancement varies with latitude (more advanced species tend to be concentrated at lower latitudes; there is a great diversity of advanced perciform fishes in the tropics and subtropics; lower euteleosts dominate at high latitudes) (McDowall, 2008). Warmer temperatures, thus, 'facilitate' the expression of plasticity.

To explain the abrupt transition, as opposed to the expected linear gradient, in the vertebrae number observed on a range of 21° of latitude, Morris *et al.* (2017) suggested a dual role of plasticity and selection intervening in Jordan's rule. They formulated the hypothesis that even if average sea surface temperatures vary linearly with latitude, near the coast there could be places where the temperature is warmer. The marine *G. aculeatus* look for an optimal range of temperature in these places to build their nests. Selection is considered lower in these microclimatic zones: waters within a range of warmer temperatures vary less in viscosity than do waters at a similar range of cold temperatures and the predator escape velocity would not be affected across a range of warm temperatures.

These contradictory pieces of evidence emerging from investigations carried out on the inter-population variation over a broad latitudinal

range highlight that changes in vertebral counts can be adaptive (through genetic selection), environmentally driven (plasticity), or a mix of both.

3.2.1 Fluctuant asymmetry

No bilateral structure is perfectly symmetrical, but in the absence of any intrinsic or extrinsic perturbations, all individuals in a sample should be perfectly symmetrical (Leary and Allendorf, 1989; Palmer and Strobeck, 1992). The lower the success of developmental stability processes in reducing the developmental noise provoked by internal or external stressors deviating from the normal developmental trajectories, the greater is the deviation from the species-specific symmetry. Developmental noise is theoretically the final effect of small environmental perturbations or accidents (Waddington, 1957; Leamy and Klingenberg, 2005) while developmental stability has long been assumed to be at least partly under genetic control (Palmer and Strobeck, 1992; Leamy and Klingenberg, 2005). The developmental stability buffers the developmental noise by canalization, namely the ability to develop the same phenotype despite genetic or environmental variability. According to the 'developmental mapping' model (see Leamy and Klingenberg, 2005), non-genetic factors and genotype interact in a non-linear function: plots of a phenotype trait against the genetic and environmental factors will be a curved slope. Non-linear developmental mapping is associated with non-additive genetic effects; thus, dominance and epistasis are expected to be the prevalent features of the genetic architecture of asymmetries. Furthermore, non-linear feedback processes may equalize the absolute variation of different structures (Leamy and Klingenberg, 2005).

Asymmetries can be assessed by morphometrics (size) or meristic (counts) changes of bilateral traits. In this chapter, only asymmetries in meristic counts will be considered. Three identifiable patterns of asymmetry variations are described in organisms: (i) *directional asymmetry* (DA); (ii) *fluctuant asymmetry* (FA); and (iii) *antisymmetry* (AS). DA, quite common, is the consistent larger value of a character on one side of a plane of symmetry; it is statistically characterized by an R-L mean not equal to zero and a normal distribution (Allanbach, 2011). AS is rarer and is present when a species character shows a larger count on one

side, but this asymmetry occurs to the right in 50% of cases and to the left in 50% of cases or is characterized by a mixture of left- and right-biased individuals (Palmer and Strobeck, 2002). Both DA and AS generally result from normal development. FA is random, independent, and a cumulative deflection for each member of a bilateral pair of characters with no tendency for one side to have a larger value than the other (Leamy and Klingenberg, 2005). FA has long been considered primarily environmental in origin and is largely used to measure developmental instability in populations (Leary and Allendorf, 1989; Palmer and Strobeck, 1992; Leamy and Klingenberg, 2005; Seixas *et al.*, 2016), under the assumption that the larger the FA, the lower is the developmental stability (Palmer and Strobeck, 1992).

Measurements of FA have since become a popular technique for quick and inexpensive examination of sublethal stress (Lutterschmidt *et al.*, 2016; Seixas *et al.*, 2016). Generally, when the existence of a genetic basis for the variability of a trait showing FA is present (i.e. broad sense heritability is greater than zero), then FA should represent a measure of the sensitivity of development to accidents. FA is influenced by:

- genetic stress (homozygosity, inbreeding, hybridization, mutation);
- extreme physical conditions (habitat changes); and
- pollution or declines in habitat quality (Palmer and Strobeck, 1992; Leamy and Klingenberg, 2005).

There is little evidence for specific genes that govern FA *per se*; some studies showed FA levels in various characters influenced by dominance and especially epistatic interactions among genes (Leamy and Klingenberg, 2005). Although the heritability of FA typically is very low or zero, epistasis can generate additive genetic variation for FA especially in populations subjected to bottlenecks, hybridizations, or periods of rapid environmental changes caused by various forms of stress.

Because of non-linear developmental mapping, different genotypes can have different developmental instability and FA (Leamy and Klingenberg, 2005) and FA is considered a population parameter as well as an individual parameter (Lutterschmidt *et al.*, 2016). Developmental differences among individuals within the same population may be strongly correlated with the temporal aspects of the environment (Lutterschmidt *et al.*, 2016).

Hechter *et al.* (2000) found that female *Culaea inconstans* with symmetrical pectoral fin rays had significantly more eggs and greater ovary weights than asymmetrical females, while more asymmetrical pectoral rays seemed to limit the range of feeding niches in *G. aculeatus* (Eric *et al.*, 2007).

There is a lack of sound evidence to indicate a clear, linear connection between FA and environmental stress. The main reason lies in the complex and mainly unknown interactions between biotic and xenobiotic factors, and these become even more complicated in a climate change scenario. Another confusing factor is the lack of FA data on unstressed populations. As far as global warming is concerned, increased FA related exclusively to variability in water temperature is reported in *Oncorhynchus keta* by Beacham (1990), in *Oncorhynchus mykiss* by Leary *et al.* (1992) and Turner *et al.* (2007), in *Gambusia holbrooki* by Mulvey *et al.* (1994), in *Oncorhynchus kisutch* by Campbell *et al.* (1998), and in *Salmo salar* by Eriksen *et al.* (2008).

3.3 Skeletal Deformations

The first evidence of skeletal deformations in wild fish (e.g. Figs 3.1 and 3.2) can be attributed to Yarrell who in 1836 classified some mullets with a short and higher-backed body, found only in rivers, as belonging to a new species, *Mugil curtus* (cited in GBIF, 2019); in reality the samples observed by Yarrell were *Chelon labrosus* with severe axis deviations and vertebral fusions. Ford and Bull (1926) reported vertebrae malformations in herring *Clupea harengus* and Kändler (1932) described extensive duplications of neural spines in flatfishes. Ninni (1933) depicted extraordinary axis deviations in *Sargus vulgaris* (now *Diplodus vulgaris*) and *Anguilla vulgaris* (now *Anguilla anguilla*) and the presence of deformed mullets in Venetian lagoons.

In a subsequent study on over 100 teleost species, Ford (1937) reported abnormal vertebrae affecting a frequency of individuals ranging from 15% (*Sardina pilchardus*) up to 59% (*Gadus merlangus*). However, the fusions of adjacent vertebrae, elongated vertebral centra and duplication of neural and haemal arches, categorized as 'complex segments', were not connected to altered environment but defined as 'hardly surprising'. In many reports, deformities have been detected in wild fish from unpolluted habitats, for example herring (Ford and Bull, 1926), *Fundulus* sp. (Gabriel, 1944), *Oncorhynchus*

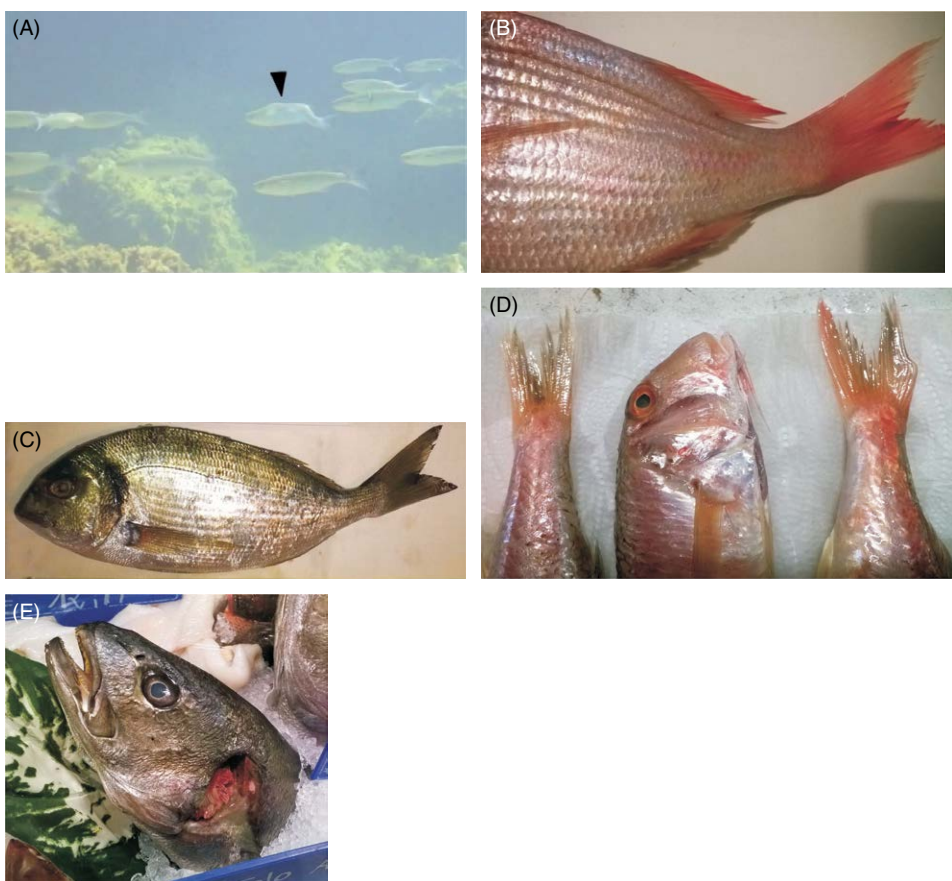


Fig. 3.1. Deformed wild brackish and marine fishes. (A) Freeze frame from an underwater video showing an adult deformed mullet (arrowhead) swimming in the north Tyrrhenian coastal waters. (B) *Pagellus affinis* showing a caudal lordosis. (C) Gilthead sea bream (*Sparus aurata*) with caudal lordosis. (D) Surmullet (*Mullus surmuletus*). The caudal fin on the right has deformed principal caudal rays. (E) Shi drum (*Umbrina cirrosa*) with extensive opercular deformation, sold as wild-caught (FAO 37) at the market. (A, courtesy of Dr C. Costa; B–E, originals by Dr C. Boglione.)

nerka, *Oncorhynchus gorbusha*, *O. keta* (Gill and Fisk, 1966), *Gadus morhua* (Fjellidal *et al.*, 2009), *Solea senegalensis* (Gavaia *et al.*, 2009) and mullets (Boglione *et al.*, 2006).

Since the first inclusion of skeletal deformities in fishes among the DELT anomalies (including deformities, eroded fins, lesions and tumours) in the Index of Biological Integrity (IBI) by Karr (1981), their role as indicators of environmental stress in water bodies has been accepted by an increasing number of authors (Boglione *et al.*, 1998, 2006; Karen *et al.*, 2001; Latif *et al.*, 2001; Pastva *et al.*, 2001; Klumpp *et al.*, 2002; Lemly, 2002; Sun *et al.*, 2009; Diggles, 2013). Other warnings of skeletal

deformities in wild fishes followed (Matsusato, 1986; Hardig *et al.*, 1988; Mayer *et al.*, 1988; O'Connor and Huggett, 1988; Haya, 1989; Weigand *et al.*, 1989; Weis and Weis, 1989; Carls and Rice, 1990; Fausch *et al.*, 1990; Lindsejöö and Thulin, 1992; Oberdoff and Hughes, 1992; Whittle *et al.*, 1992; Boglione *et al.*, 1993, 1994, 2001, 2003; Da Cunha and Antunes, 1999; Ferreri *et al.*, 2000; Tutman *et al.*, 2000; Antunes and Da Cunha, 2002; Dulçiç, 2004; Favalaro and Mazzola, 2006; Ayed *et al.*, 2008; Fjellidal *et al.*, 2009; Gavaia *et al.*, 2009; Diggles, 2013; Sfakianakis *et al.*, 2013; Pollock, 2015; Jawad and Ibrahim, 2017; Jawad *et al.*, 2017).

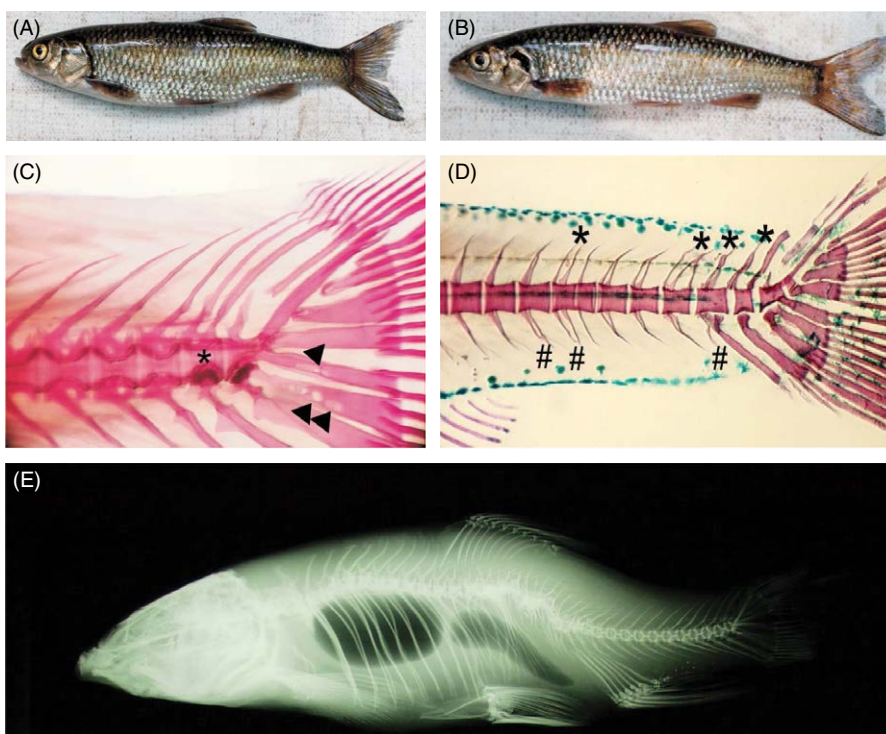


Fig. 3.2. Deformed wild freshwater fishes. (A) Chub (*Squalius squalus*) with pugheadness (reduced premaxillary) and crossbite. (B) Chub (*S. squalus*) with reduced opercular plate. (C) Deformed caudal vertebra (*) carrying two neural arches in a juvenile chub (*S. squalus*). Note the presence of some decalcified areas (arrowheads) in the parahypural and hypural. *In toto* staining with Alizarina red. (D) *Sarmarutilus rubilio* (Cyprinidae) post-larva showing deformed neural (*) and haemal (#) arches. Note the differences existing in size of the vertebrae. *In toto* staining with Alizarina red. (E) Radiograph of an adult *Barbus plebejus* (Cyprinidae) with severe and multiple axis deviation, vertebrae deformations and head anomaly. (A and B, courtesy of Dr L. Tancioni; C–E, originals by Dr C. Boglione.)

3.3.1 Main typologies of skeletal malformations in wild fishes

The notochord is the first skeletal tissue to differentiate in the embryo and in early life stages. It plays both structural and patterning roles as early notochord signals influence the cell-fate and patterning in the spinal cord and in somites. Merckel's cartilage and cleithrum are the first cartilaginous and bone tissues to differentiate soon after. The information on occurrences of malformed wild embryos is quite rare and almost entirely limited to marine species. Some species-specific differences in occurrence of deformation rates in embryos have been described with higher occurrences at locations nearer the shore than farther out (Cameron *et al.*, 1992; Cameron and von Westernhagen, 1997; von Westernhagen and Dethlefsen, 1997). Deformities

in embryos are generally lethal. The 85% of the registered malformations in flatfish embryos and early larvae were lethal within 5 days according to Cameron *et al.* (1992). Many authors consider embryological analyses as a sensitive, practical and relevant means of monitoring environmental quality. However, the reported embryonic anomalies are mainly those affecting very early stages, before the tail-bud stage, when no skeletal structures are yet differentiated. In general, notochordal defects are quite rarely described in later embryos because with progressive development, malformations decreased in numbers, as a result of the embryos' decreasing sensitivity with ongoing development and the loss of aberrant embryos due to selective mortality (von Westernhagen *et al.*, 1988; Cameron *et al.*, 1992; Cameron and von Westernhagen, 1997; von Westernhagen and Dethlefsen, 1997).

Some bent notochord (von Westernhagen *et al.*, 1988; Cameron *et al.*, 1992; Cameron and von Westernhagen, 1997), twisting of the tail, deformation of the head region and finfold abnormalities (Cameron *et al.*, 1992) have been described in pre-hatching embryos or just-hatched larvae from polluted areas. Anthropogenic pollutants are suspected to be the highest potential factor to contribute to these early deformities, acting through the accumulation of toxicants in the parental gonads and/or by effects on the embryo through the incorporated substances.

In older stages and in adults, each skeletal element can be indiscriminately stricken with deformation, although most of the literature reports record deformed vertebral columns (lordosis, kyphosis and scoliosis, even in association) and/or vertebrae (centra and arches) in wild fish (Ninni, 1933; Grimaldi, 1965; Gill and Fisk, 1966; Amin, 1968; Patten, 1968; Kroger and Guthrie, 1971; Balbontin *et al.*, 1973; Scherer, 1973; Cavaliere *et al.*, 1975; Moore and Hixons, 1977; Berra and Au, 1981; Sloof, 1982; Matsusato, 1986; Langdon, 1987; Matsuoka, 1987, 2003; Francescon *et al.*, 1988; Honma, 1989; Loganathan *et al.*, 1989; Van den Avyle *et al.*, 1989; Pohl, 1990; Purcell *et al.*, 1990; Williamson *et al.*, 1991; Boglione *et al.*, 1994, 1998, 2001, 2006; Couillard *et al.*, 1997; Da Cunha and Antunes, 1999; Ferreri *et al.*, 2000; Tutman *et al.*, 2000; Antunes and Da Cunha, 2002; Dulçic, 2004; Favalaro and Mazzola, 2006; Ayed *et al.*, 2008; Tesch *et al.*, 2008; Fjellidal *et al.*, 2009; Gavaia *et al.*, 2009) (Fig. 3.1A–C and Fig. 3.2C–E).

Head deformities also occur mainly affecting the splanchnocranium rather than the visceral cranium: pugheadness, cross-bite (Fig. 3.2A) and lower jaw reduction or elongation are the most common deformities found in freshwater and marine finfish (Lindesjoo and Thulin, 1992; Boglione *et al.*, 2001, 2006; Tesch *et al.*, 2008; Jawad and Ibrahim, 2017), sometimes in association with vertebral axis distortions (Honma, 1989). Opercular deformations (Fig. 3.1E, Fig. 3.2B) are quite rare in wild fishes (Valentine and Bridges, 1969; Sloof, 1982; Boglione *et al.*, 2001, 2006; Jawad and Ibrahim, 2017); a resulting theory is that some of the wild fishes affected may be reared fish that have escaped from fish-farm cages (Boglione *et al.*, 2003; see Fig. 3.1E).

The reporting of fin deformations (Fig. 3.1D) (malformed rays of pterygiophores, eteroptery, aptery) and of predorsal/supraneural/interdorsal bones is less common than deformations in the vertebrae (Valentine

and Bridges, 1969; Dahlberg, 1970; Scherer, 1973; Sloof, 1982; Williamson *et al.*, 1991; Ferreri *et al.*, 2000; Valladolid and Przybylski, 2000; Boglione *et al.*, 2001, 2006; Matsuoka, 2003; Favalaro and Mazzola, 2006; Gavaia *et al.*, 2009; Jawad and Ibrahim, 2017). Recently, saddleback malformation has been increasingly reported in wild marine fish. It consists of the complete loss of pterygiophores and rays of dorsal fin, sometimes associated with deformity, disarrangement and shortening of the neural spines below the dorsal fins, and of the supraneural bones anterior to the dorsal fin (Berra and Au, 1981; Diggles, 2013; Jawad and Ibrahim, 2017; Jawad *et al.*, 2017). However, in some cases it has been considered a scar from physical injuries experienced at the juvenile stage (Pollock, 2015).

3.3.2 Effects of skeletal malformations on fish performance

Skeleton deformities have been described in both wild juveniles and adults. According to Koumoundouros *et al.* (2001), it is reasonable to expect that the natural environment imparts severe selection pressure on deformed individuals; however, the presence of deformed wild adult fishes means that some deformed juveniles can survive into adulthood (Diggles, 2013) or that they may contract deformities during the adult stage.

There is little literature on the effects of skeletal anomalies on biological performance of wild fish. The main body of information is limited to deformed fish from aquaculture, where they represent one of the main bottlenecks towards massive production of commercial fishes. The gravity of the downgrade of the biological performances due to skeletal anomalies will depend on the skeletal elements involved.

In reared *Dicentrarchus labrax* post-larvae, kyphosis (inverted V-shaped deviation of the vertebral column) in the abdominal vertebrae induced lethargic behaviour and a subsequent heavy mortality in the following stages, resulting in compression of the neural tube by the deformed vertebrae (Koumoundouros *et al.*, 2002a). According to Basaran *et al.* (2007) lordosis (V-shaped deviation of the vertebral column) significantly decreases the endurance and critical swimming speed of *D. labrax* juveniles. In general, seriously deformed vertebral arches can affect blood flow and spinal cord function. In *C. harengus*, the spinal condition was the most important predictor of swimming ability

while the lower jaw size was the second predictor in post-larvae hatched from eggs experimentally exposed to 0.7 ppb polynuclear aromatic hydrocarbons (PAHs) (Carls *et al.*, 1999).

Selective mortality for deformed fish in farming conditions has been described. Puvanendran *et al.* (2009) observed a decline in larval deformity from 22% at hatch to 8% at 14 days post hatching (dph) and to 2.5% at 42 dph in *G. morhua* while Koumoundouros *et al.* (2002a) and Dou *et al.* (2005) found that most dead post-larvae had jaw and vertebral deformities. Serious jaw anomalies impair efficiency in feeding with negative consequences on the growth rate.

Gill cover anomalies have been shown to increase the sensitivity to oxygen stress and a predisposition to myxobacterial infections (Paperna *et al.*, 1981), as branchial mucosae, rich in blood vessels, are unprotected by the opercular plate and so are directly exposed to pathogens. In farming conditions, opercular plate deformations in *Sparus aurata* post-larvae negatively correlated with the growth rate (Koumoundouros *et al.*, 1997). Their incidence showed a 2.5–4.0-fold decrease during the metamorphosis phase, an indication of the possibility of a selective mortality of the deformed fish (Georgakopoulou *et al.*, 2010). Abdel *et al.* (2004) found lower average weight of reared *D. labrax* with operculum bilateral abnormalities than in normal fish.

Anomalies affecting the fins may induce negative effects on fish locomotion with effects varying according to the involved fin, the seriousness of the anomalies and species-specific swimming and feeding behaviour. The absence of the anterior half of the dorsal fin in reared *Oreochromis aurea* affected by saddleback syndrome has no noticeable impact on swimming behaviour, but when most or all of the dorsal fin is missing, fish have difficulty maintaining equilibrium during swimming (Tave *et al.*, 1983). Poor swimming performance would be selectively disadvantageous to larval and juvenile fishes, as it would increase their chances of predation, and reduce their own prey capture ability. The deformations are imputed to cause delay in growth and high mortality rates during any stress situation (Abdel *et al.*, 2004).

Any wild larval or early juvenile fish with severe deformities could not be expected to survive; they would never reach a size where they would be recruited into the fishery and be detected (Diggles, 2013). Consequently, occurrences of deformed

adult fishes should be considered as the ‘tip of the iceberg’, representing the few surviving deformed post-larvae or juveniles (Diggles, 2013).

However, taking into consideration that each skeletal tissue undergoes differentiation, trans-differentiation, modelling and remodelling along the entire life cycle in fish, the possibility that they can become deformed in adulthood should not be excluded. In aquaculture conditions, some vertebrae fusion and related anomalies have been proved to develop into subadults (e.g. after smoltification in Atlantic salmon; Witten *et al.*, 2005).

3.3.3 Main causes of skeletal malformations in wild fish

The literature on skeletal deformations in wild fish rarely indicates a clear cause–deformity relationship, for the existence of many single and/or multiple factors, often acting in synergy, that provoke stress in skeleton-related processes, including: (i) abiotic factors (temperature, salinity, pH, O₂, CO₂, light intensity); (ii) biotic components (genetic, food availability and quality, space limitation, changes in swimming modality, water velocity, predation, bacteria, viruses, parasites); and (iii) xenobiotic components in the water column and/or in sediments. Both biomechanical and biomolecular processes, in fact, act on differentiation, growth and repair of the fish skeleton.

The research applied to improving the morphological quality of fish produced in aquaculture has highlighted that different stressors can produce the same anomaly in different species but that the effects of a single biotic or abiotic stressor (i.e. temperature, stress, nutrition factor) may be different according to the fish species (Boglione and Costa, 2011), or the life stage (Mazurais *et al.*, 2009). Moreover, the same stressor may act on a different part of the skeleton in different species (Koumoundouros, 2010) while in the same fish species it can provoke a higher malformation rate in some skeletal elements but not in others, even if sharing the same skeletal tissue and ossification process (Fernández and Gisbert, 2011). In addition, several stressors can act on different cohorts of the same species (Kause *et al.*, 2007).

Incubation temperatures higher than the species-specific range of tolerance induce higher occurrences of skeletal deformities in cold- (i.e. in salmonids: Murray and Beacham, 1986; Stickland *et al.*, 1988; Wargelius *et al.*, 2010; in Atlantic cod: Fitzsimmons and Perutz, 2006; in wolfish, *Anarhichas lupus*:

Pavlov and Moksness, 1994) as well as warm-water reared species (Senegalese sole: Dionísio *et al.*, 2012; zebrafish: Pype *et al.*, 2015; *Trachinotus ovatus*: Ma *et al.*, 2016; sea bass: Abdel *et al.*, 2004; Sfakianakis *et al.*, 2006; gilthead sea bream: Georgakopoulou *et al.*, 2010). Higher temperatures and hypoxia during embryonic stages correlated with anomalous vertebral formation in Atlantic salmon (Wargelius *et al.*, 2005) and in red sea bream *Pagrus major* (Sawada *et al.*, 2006). Hypoxia caused increased rates of deformities in *Seriola dumerili* (Sawada *et al.*, 2006) and *Acanthopagrus butcheri* (Hassell *et al.*, 2008). Warmer temperatures and hypercapnic conditions determined a threefold augmentation of deformity rates in gilthead sea bream (Pimentel *et al.*, 2016).

Higher salinity was shown to cause skeletal deformities (Haddy and Pankhurst, 2000; Cobcroft *et al.*, 2001; Sfakianakis *et al.*, 2006; Fernández and Gisbert, 2010). Other environmental factors identified in aquaculture conditions which determine higher deformation rates are light intensity (Cobcroft *et al.*, 2001; Sfakianakis *et al.*, 2006), light regime (Fjelldal *et al.*, 2005; Wargelius *et al.*, 2009) and food quality. In aquaculture, larval nutrition has been recognized as one of the key parameters affecting skeletogenesis during early fish development (for a comprehensive review on different nutritional requirements in reared finfish larvae see Hamre *et al.*, 2013, and Rønnestad *et al.*, 2013).

Also, inappropriate hydrodynamic conditions have been linked to vertebral column malformations (particularly haemal lordosis) because they induce forced swimming (e.g. in sea bream: Andrades *et al.*, 1996; in sea bass: Divanach *et al.*, 1997; in red sea bream: Kihara *et al.*, 2002; in Atlantic cod: Baeverfjord *et al.*, 2009). The bone matrix developed during sustained swimming has been proved to have significantly higher mineral content and mechanical strength in salmon, an adaptive response to strains produced in response to mechanical loading probably mediated by osteoblasts or bone lining cells in fish (Totland *et al.*, 2011). The osteocyte density in the exercised fish increased significantly in the compact lamellar bone of the amphicoel: the mechanical stimulation in this layer may thus have activated osteoblasts and tuned the genetic pathways regulating the protein secretion (i.e. *bglp* and *alp*) that determines the mineral formation process. In mammals, *alp* seems to provide a high PO_4 concentration at the osteoblast cell surface during the formation of hydroxyapatite crystals while *bglp* may coordinate calcium-binding

properties probably involved in the regulation of bone growth (Totland *et al.*, 2011). Cloutier *et al.* (2010) demonstrated that the sequence of chondrification and ossification in larvae and post-larvae of *Salvelinus alpinus* proved to be modified by water velocity: ossification was more responsive to water velocity than chondrification, and early-forming elements less responsive than late-forming elements. In addition a faster sustained swimming (behavioural adaptation to a higher water velocity) could induce differential mechanical stresses on developing skeletal elements involved in locomotion and induce anomalies (e.g. fusion, additional elements) in dorsal and anal fins.

Ecotoxicological studies identified some chemical substances that increase spinal deformities in fishes, for example: (i) diuron (Gagnon and Rawson, 2009); (ii) kepone and trifluralin (Couch *et al.*, 1977, 1979); (iii) cadmium (Pragtheeswaran *et al.*, 1987); (iv) selenium (Lemly, 2002; Teh *et al.*, 2002; Hamilton *et al.*, 2005; Muscatello *et al.*, 2006); and (v) zinc and cadmium (Mehrlé *et al.*, 1982; Sloof, 1982; Pohl, 1990). Synergistic effects between temperature and heavy metal mobility cause spinal deformities in mosquitofish (Sassi *et al.*, 2010). De Andrade Brito *et al.* (2018) reported that some PAHs (chrysene and benz[a]anthracene) were associated with deformities of the spine and cranium in *Rhamdia quelen*, but the authors could not exclude that other contaminants in the water could also have been responsible for the toxicity. Fernández *et al.* (2017) reviewed the osteotoxicity of more than 40 different toxicants in six different fish systems (larvae, juvenile and adult stages, cell culture, scales and caudal fin regeneration). The reported osteotoxic effects were impaired chondroblast, osteoblast and/or osteoclast differentiation and activity, altered extracellular matrix mineralization/composition and/or physical properties, increased rate of skeletal deformities, and loss/gain of particular skeletal structures. Zebrafish exposed to moderate-to-high levels of polychlorinated biphenyls (PCBs) during the early developmental stages exhibited increased skeletal malformations and calcium loss (Ju *et al.*, 2012). Skeleton deformities found in one population of *Tor putitora* in Dhobi Ghat Stream in proximity of a laundry wastewater were associated with detergents by Majeed *et al.* (2018). PCBs, cadmium and lead also could weaken vertebral structures (Mehrlé *et al.*, 1982). Sun *et al.* (2009) described a significant relationship between split fins, lower jaw protrusion and gill deformities with

low dissolved oxygen and high ammonium, lead and zinc concentrations.

Recent studies have been conducted on the possible involvement of genetic drift, mutations, inbreeding, selective breeding and polyploidy in skeletal deformation in farmed fishes. In general, the genetic factors seem to influence only some vertebral axis deviations, particularly when they are multiple (i.e. kypho-lordo-kypnosis) (Afonso *et al.*, 2000; Gjerde *et al.*, 2005; Thorland *et al.*, 2007; Bardon *et al.*, 2009; Negrín-Báez *et al.*, 2015; García-Celdrán *et al.*, 2016). However, the main body of data seems to indicate that if a genetic basis for skeletal anomalies is observed, this predisposition is expressed only under exceptional environmental conditions (e.g. energy failure, rising temperature) and that the susceptibility for the presence/absence of vertebral column anomalies has a high additive genetic component (Kolstad *et al.*, 2006; Kause *et al.*, 2007).

3.4 Effects of Global Warming on Skeletal Anomalies

Ongoing rising temperatures involve alterations of many water features that can directly or indirectly affect bone modelling and remodelling processes in the fish skeleton. These alterations may alter chemical, physical and/or biomolecular properties, and pattern and sequence of skeletal processes. These effects can vary in magnitude among fish living in salt, brackish or fresh waters, among those inhabiting open ocean, coastal zones or continental waters, between fish species evolutionarily adapted to live in cold (stenothermal) or warmer (eurythermal) water, in deep or surface waters, and among the different life stages (further details and references in the following sections).

Global warming determines abiotic changes (temperature, oxygen, CO₂, salinity, pH, metals and toxicant mobility and bioavailability) inducing indirect biotic modifications (physiological, ecological, behavioural), each possibly interfering with skeleton-related processes.

3.4.1 Direct effects of warmer temperature

Fish are ectothermic vertebrates with limited thermal acclimatization of metabolism; recently the role played by thyroid hormones (TH), viz. 3,5,30-triiodothyronine (T₃) and 3,5-diiodothyronine (T₂) has been described in zebrafish (Little *et al.*, 2013). The actions of TH in zebrafish are temperature specific,

with lower sensitivity at warm than at cold temperatures; inductive or repressive responses also depend on the animal's thermal history. The low sensitivity of TH at warm temperatures could mean that increasing temperatures would reduce the capacity of zebrafish to regulate their energy metabolism and locomotory performance. This should be added to the temperature effect on bioavailability and toxicity of thyroid-disrupting pollutants, whose levels are increasing worldwide. TH levels influence skeletogenic processes: Bolotovskiy and Levin (2018) demonstrated in freshwater and brackish cyprinids that manipulated thyroid gland can induce developmental heterochronies accountable for changes in the number of scales, fin rays, pharyngeal teeth and total number (mostly caudal) of vertebrae. As noted above, changes in the number of fin rays and of the different vertebrae influences the fishes' locomotory ability.

The heat shock proteins (HSP) are a family of molecular chaperones, which mediate the protein folding pathway and a number of HSP genes are expressed at high levels during embryonic development in fish. During exposure to elevated temperature or other environmental stresses (environmental contaminants included), HSP genes are activated, enhancing HSP synthesis as protection from cellular damage (Karouna-Renier and Zehr, 1999). HSP proteins influence muscle differentiation and skeleton differentiation and regeneration. Detectable levels of the inducible isoform of HSP70 were demonstrated after acute stressor insults in fish. Recently (Bertotto *et al.*, 2011) detected correlated expression of HSP70 with insulin-like growth factor I (IGF-I) and myostatin (MSTN) mRNAs in the early development of sea bass. Even HSP90 plays some role in skeletal myogenesis: the expression of HSP90a is restricted predominantly to myoD-expressing cells in pre-somitic paraxial mesoderm, somites and pectoral fin buds of developing zebrafish embryos (Krone *et al.*, 1997). Lele and Krone (1997) describe that HSP47 mRNA activity is predominantly with expression of the type II collagen gene (*col2a1*) in a number of chondrogenic and non-chondrogenic tissues, including the notochord and developing fins as demonstrated in zebrafish. More recently Bhadra and Iovine (2015) demonstrated that knockdown of HSP47 in zebrafish regenerating fins provoked reduced fin and segment length and lower cell proliferation, as well as affecting organization and localization of the collagen-based actinotrichia.

The solubility of oxygen decreases as temperature increases and the metabolic rate of all ectothermic organisms is strongly dependent on temperature (as well as body size). Thus, rising temperatures accelerate metabolic rate and alter aerobic physiology. This means that the resting metabolic rate (minimum energy required at rest) increases due to the inability of the circulatory and ventilatory systems to tackle the increased oxygen demand. Consequently, aerobic capacity could be weakened when controlling both non-essential activities (swimming, foraging, growth and energy storage) and higher-level performances (see Donelson 2015 for more details and references). Loizides *et al.* (2014) showed that elevated water temperature induces a shift of the ontogenetic timing of *S. aurata* towards smaller body sizes at fin formation and squamation. Nonetheless, some species supposedly evolutionarily acclimatized to warmer temperatures could perform better across different temperature conditions due to some 'overcompensation of metabolic attributes' (*sensu* Donelson, 2015); this makes the acclimatization response to future warming more complex than expected. Higher growth rates have been observed in a coral reef fish, *Premnas biaculeatus*, reared at +3.0°C (Donelson, 2015). Laurence (1978) described a specific growth rate increased by two to three times over a 5°C range from hatching to metamorphosis in *G. morhua* and *Melanogrammus aeglefinus*, in laboratory conditions. Frairner *et al.* (2017) detected that the recent warming period in the Barents Sea triggered a rapid shift from an Arctic fish community characterized by small-sized bottom-dwelling benthivores, towards a boreal fish community characterized by large body size (and longer lifespan, and piscivory). Larger body size is associated with changes in muscle fibre. De Mello *et al.* (2016) found a positive correlation between body weight and muscle fibre diameter. Koumoundouros *et al.* (2009) reported a significantly higher swimming capacity in juvenile European sea bass at 15°C during larval rearing than for the fish initially reared at 20°C. Such higher performances were associated with increased muscle fibre types. Furthermore, muscle fibre type, mitochondrial density, muscle mass and body shape have all been shown to exhibit plasticity in response to temperature (Oufiero and Whitlow, 2016).

Temperature significantly affects swimming performance due to modifications of the molecular kinetics and the rates of the biochemical reactions

(that convert chemical energy into propulsive thrust); it also changes the physical properties of the water, which in turn affects the fishes' movements (Koumoundouros *et al.*, 2002b). The U_{crit} (critical swimming speed, a measure of prolonged exercise, mainly aerobic swimming performance, which is terminated by fish exhaustion) changes according to water temperature, with the highest values at the optimum and decreasing at the upper or lower thermal ranges. Allen *et al.* (2006) found that the U_{crit} of juvenile *Acipenser medirostris* increased with acclimatization to higher temperatures (from 19°C up to 24°C), concomitant with an increasing of six HSPs in muscle and/or pelvic fin. According to Stevens (1979), kinematic changes due to temperature vary according to species: *O. mykiss* had lower tail beat frequencies at increased temperatures (5°C versus 15°C) while *Micropterus salmoides* showed an increase with increased temperature (tested temperatures: 12°C, 25°C and 30°C). Augmented tail beats and amplitude increase the power output and swimming speed (Oufiero *et al.*, 2014).

Water temperature also influences viscosity, one of the two forces, together with pressure, acting on a moving object in fluids. If viscosity diminishes, then the ratio of inertial forces to viscous forces (Reynolds number, Re) will augment: the water flow changes from turbulent to laminar and swimming from resistive to inertial. Thus, the energy cost (i.e. the oxygen demand) and effectiveness (U_{crit}) of larval fish movements change according to the Reynolds number (Re). Fish larvae spend 98% of time cruising (normal swimming) in viscous (Re < 30) and intermediate flows (30 < Re < 200), in conditions where gliding is very ineffective due the rapid loss of the kinetic energy for counteracting friction. Even the first part of burst swimming (escape responses) is in the viscous and later in the intermediate regime (Osse and Van den Boogaart, 1995). Further, at low Re values, prevalent swimming movements are anguilliform, while at higher Re numbers subcarangiform swimming movements prevail (Webb and Weihs, 1986). Thus, as the temperature rises, water loses viscosity (and Re value augments) while surface tension (thus oxygen concentration) decreases. When fish face augmented Re values, it is expected that they will swim easily, with more effective gliding, in routine behaviour (Danos and Staab, 2010) with turning from prevalent anguilliform towards subcarangiform movements (Webb and Weihs, 1986). Swimming in warmer (low viscosity) water requires smaller muscular

force, viz. a lower recruitment of fast anaerobic muscle fibres and an augmented power output of aerobic muscle fibre types. All these changes in muscle fibres, body weight and locomotion induced by temperature have been shown to induce differential mechanical stress, and varying pressure on skeletal elements, which may provoke change or anomalies in bone shape, size or toughness. The vertebrate skeleton responds to mechanical forces: suitable mechanical stimulation is required for the maintenance of healthy bones, for bone repair and for osteogenesis. Faster growth rates and heavier body weights at larval and juvenile stages affect the regulatory interactions between muscle pressure and the underlying differentiating skeleton. It is recognized that external mechanical forces regulate genetic pathways of both cartilage and bone development and the skeletal tissues are capable of adapting their structures, shape and mechanical features in response to altered loading conditions in teleost fish (see Boglione *et al.*, 2013). In fish, bone responds preferentially to dynamic stimuli rather than to static ones; indeed, even very short loading durations can induce an adaptive response. This implies that skeleton development and growth could be negatively influenced by heavier body weight (namely heavier mechanic compression) or by changed swimming modalities due to changing viscosity or reduced space availability.

Also, rising temperature affects the number (pleomerism) and identity (homeosis) of vertebrae, both influencing the fish's swimming modality. For example, the number of vertebrae influences axial flexibility; this can influence maneuverability, burst acceleration and cruising speed (Reimchen and Cox, 2016). The costs for tackling viscosity changes are believed to be higher for cold-water species, in particular polar (Notothenioid) fishes, where the energy used for growth at low temperature is compensated by savings at cellular and whole organism levels (reduced ion exchange activities, large myocytes in cardiac and skeletal muscle, reduction of bones, reduced calcification of the skeleton, gill raker, fin and body sizes, use of lipid body stores for neutral buoyancy, low activity lifestyles) (as reviewed by Pörtner, 2006). Thus, these cold-adapted fishes may find themselves with a skeleton unfit for less viscous water; their swimming performances at higher temperatures could be reduced, and a forced unnatural style of swimming could possibly lead to a higher risk of developing skeletal anomalies.

In fish, a correlation between vertebral phenotype and behavioural temperature preference serves to maintain swimming efficiency during migration, foraging or evasive responses to predators. Rising atmospheric temperature causes higher temperatures on the surface waters than in deep waters. Pelagic species may be more sensitive to future environmental changes since they are adapted to a more stable environment, compared with demersal species that regularly experience fluctuating environmental conditions.

In addition, stratification in a body of water can increase with warmer temperature, with consequent suppression of the mixing of nutrients in the euphotic zone and decreasing of the fertilizing effects of mixing and upwelling. Consequently, the fish should face two choices: (i) changing from pelagic to benthic habits; or (ii) reduce their time in superficial waters at minimum. Both strategies involve some energy costs and cause changes in swimming modality which are unnatural for many species (e.g. continuous vertical migrations in salmonids as observed in cage-reared Atlantic salmon; author's personal observations). In this case, upward movements are due to the search for food in the warmer euphotic surface waters, while downward movement is caused by the fishes having to plunge into the colder strata to escape staying too long in over-warmed temperatures. Furthermore, deeper water in a strongly stratified sea may determine the formation of anoxic deep layers, thus limiting survival of deep-water fishes.

High water temperature accelerates differentiation and growth rates, resulting in a significantly shorter time spent in the larval niche and a possible significant earlier settlement of pelagic larvae in shallow coastal nursery grounds. This accelerated differentiation time is accompanied by an earlier attainment of swimming capabilities. Some reports highlight how increasing temperature provokes some variation in the sequence and rate of ossification (e.g. in zebrafish and in *Betta splendens*; in *Oreochromis mossambicus*), and deformities in axial and appendicular skeleton (*Anarhichas lupus*) (see citations in Cloutier *et al.*, 2010). Chondrogenesis, chondrification and ossification are the main skeletogenic processes in fish, their coordinated patterns and sequences being functionally relevant. The persistence of the chondrogenic process and a delay in the osteogenic one during larval development could evolve into discrete heterochronic shifts in skeletal gene expression affecting the allometric

growth of the different elements, as well as developmental asynchronies, leading to skeletal deformations and impaired functionality. Consequently, precocious settlement of juveniles with developmental asynchronies may alter their capability to feed and escape correctly. Ytteborg *et al.* (2010) reported in Atlantic salmon that warmer (16°C versus 10°C) temperatures induced fast growth and severely affected gene transcription in osteoblasts and chondrocytes; a disrupted bone and cartilage production was detected, most likely causing the higher rate of deformities developed. In the same species, Fraser *et al.* (2015) reported an increased prevalence of vertebral deformities in larvae that experienced higher egg-incubation temperatures. Ma *et al.* (2016) reported a significantly faster growth rate, higher jaw deformity, and the lowest expression of bone morphogenetic protein (BMP)₂ and the highest expression of BMP₄ in *T. ovatus* larvae reared at higher temperature (33°C).

3.4.2 Indirect effects of warmer temperature

Increased pH, lower dissolved oxygen, alteration of nutrient recycling, and of water currents and salinity are expected to induce changes in the temporal and spatial distribution of species and lead to a reduction in species richness, even in shallow waters. The skeleton plasticity is able to modify single skeletal elements differently according to:

- the type of bone (acellular versus cellular bone);
- life style (active swimmers versus poor swimmers); and
- the nature of the aquatic environment that the fish inhabit (seawater versus fresh water).

It is also able to respond quickly to the scarcity of limiting nutrients (phosphorus in fresh water and nitrogen in seawater), oxygen, lowering pH, higher mobility of heavy metals (e.g. cadmium) and toxicity of chemical pollutants.

The impact on oceanic superficial and deep currents prefigures negative changes in migratory patterns and/or swimming modality in some fishes. Changes in water velocity, as well in water viscosity or in swimming modalities of fish are expected to augment skeletal anomalies, albeit the rate is unpredictable depending on life stage, life history and species.

Nutritional deficiencies can be the consequence of the reduced upwelling of nutrients from deep water to the euphotic zone because of stratification. This reduces primary production and the rate of

sinking of particulate organic carbon below the euphotic zone (Rogers, 2015). Subsequently, fish at every life stage will have to face some change in prey availability, breeders included, with maternal consequences on the vitelline reserves for embryos. Nutritional deficiencies enhance the frequency and gravity of skeletal deformations.

One of the main indirect effects of rising water temperature is the lowering oxygen concentration. This situation can be aggravated by stratification between warmer superficial and colder deeper water layers, provoking expansion of extreme oxygen minimum zones (eOMZs) and alteration in the relative rates of autotrophic carbon fixation versus heterotrophic remineralization (Rogers, 2015). Oxygen concentrations may be further reduced by microbial communities. Fish with high metabolic rates and oxygen requirements will face a reduced space availability between the deeper eOMZs and the warmer superficial waters whose oxygen concentration, however, is lower during the day than the night. Thus, other behavioural changes are expected due to the necessity of continuous diurnal vertical migrations.

As CO₂ concentrations rise and/or oxygen supply decreases, the scope for aerobic performance decreases across a range of temperatures (Rogers, 2015). Hypoxic conditions produce vertebral column deformities (e.g. in salmon; Sánchez *et al.*, 2011). Ocean carbonate chemistry is the third factor which dramatically influences the optimal performance of an organism. The rising atmospheric CO₂ could cause dramatic changes in organisms and in ocean physical features, hypercapnia in the body fluids, interferences with the exchange of oxygen between organisms and the environment, and ocean acidification. The chronic respiratory acidosis provoked by exposure to hypercapnic conditions results in a compensatory release of skeletal calcium and phosphorus to maintain the homeostatic blood pH, a compensatory mechanism possibly causing skeletal deformities in fish, as demonstrated by Hafs *et al.* (2012) in rainbow trout. The incidence of body deformations in *S. aurata* post-larvae more than tripled under warmer and hypercapnic conditions (Pimentel *et al.*, 2016). Elsadin *et al.* (2018) found significantly reduced swim bladder volume and body weights in *Epinephelus aeneus* juveniles reared in water with high dissolved CO₂ concentrations. Fish with reduced swim bladders have significantly higher prevalence of lordosis and kyphosis than those with normally inflated swim bladders.

The absorption of higher concentrations of atmospheric CO₂ by seawater and its conversion to carbonic acid is calculated to result in a decrease of the pH of the surface oceans by 0.3–0.4 and an increase in hydrogen ions of 100–150% (Rogers, 2015). Additionally, increases in freshwater inputs can further reduce carbonate saturation through decreases in salinity, which exacerbate ocean acidification. The increases in temperature and acidification are expected to influence both the worldwide distribution of chemicals and their toxicological effects on organisms (Noyes and Lema, 2015). Climate change–contaminant interactions may alter the bioaccumulation of two classes of priority contaminants: (i) the fat-soluble persistent organic pollutants (POPs), such as PCBs; and (ii) the protein-binding methylmercury (Alava *et al.*, 2017). Also, an increasing trend was observed with increasing water temperature for many pesticides (Patra *et al.*, 2015; Yabuki *et al.*, 2016). Even metal (copper, cadmium, zinc, selenium) mobility, forms and bioavailability (and their biotoxicity) from contaminated sediment to overlying water is increased due to acidification (Wang *et al.*, 2017). These contaminants provoke some deformation in fish skeletons. Mineralogenic, osteogenic and skeletogenic effects were observed in several fish systems (*in vitro* cell systems, developing zebrafish larvae and regenerating caudal fin), upon exposure to 3-methylcholanthrene (3-MC). This polycyclic aromatic hydrocarbon's osteotoxicity was associated with AhR (aryl hydrocarbon receptor) whose activation influences calcification (*viz.* bone matrix mineralization) and would determine the onset of skeletal deformities (Fernández *et al.*, 2017). Sassi *et al.* (2010) describe how combined exposure to Cd and high temperature led to a more significant increase in Cd accumulation and in the frequency of spinal deformities in mosquitofish than exposure to Cd or high temperature alone. Desai *et al.* (2002) reported adverse effects of Zn²⁺ on the cytoplasmic proteins of skeleton cells in fishes.

Change in the biotoxicity of pollutants driven by rising temperature could also indirectly induce skeletal anomalies: Little and Seebacher (2015) observed impaired swimming performance in zebrafish exposed to relevant concentrations (20 mg/l) of bisphenol A, a ubiquitous pollutant.

3.5 Concluding Remarks

According to the beneficial acclimation hypothesis (BAH), first articulated by Leroi *et al.* (1994),

‘acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment’.

The above highlights the different responses of fishes to warming temperature according to their autoecology, ecophysiology, evolutionary history, life history and stage. The links between physical and biological systems are non-linear; consequently, projections of changes on aquatic ecosystems have produced mixed results and are debatable. Some researchers predict a global decrease in primary production while others predict an increase (O'Brien *et al.*, 2016). Synergism makes the combined effects of multiple stressors greater than the sum of individual effects. Meanwhile research into multiple abiotic stressors and different life stages is still lacking. Some lenitive or negative synergistic effects have been noted: for example, Wang *et al.* (2017) reported that ocean acidification seems to mitigate mercury toxicity to the marine copepod *Tigriopus japonicus* under multigenerational exposures. However, Zeng *et al.* (2015) highlight a likely facilitative effect between ocean acidification and pollution: the larger potential of acidification from pollution-affected respiration with respect to the uptake of anthropogenic carbon dioxide makes coastal waters more vulnerable to the negative impact of ocean acidification than open oceans due to the large influx of pollutants from terrestrial ecosystems. The overcompensation of metabolic attributes in some species (Donelson, 2015) does not match the BAH, since not all fish performed best at their acclimatization temperature (Angilletta, 2009). The acclimatization response of species to future warming will be complex, and may not always be directed towards reduced performances (Donelson, 2015). Stressful and extreme environments can be important in promoting plasticity and adaptation (Badyaev, 2005) but species differences in physiology and costs of plasticity may modulate the ability to exhibit plasticity in different species (Donelson, 2015). Some authors report a higher sensitivity of tropical species to projected environmental warming (e.g. Donelson, 2015) because of their evolutionary adaptation to reductions and shifts in distribution range and/or the constrained persistence at present locations. Conversely, others (e.g. Wells, 2009) predict more negative effects on Antarctic than Arctic or eurythermal species, because of their greater capacity for acclimatization. Even

the thermal plasticity in swimming performance could change according to species, and among different populations; all changes in individual traits due to temperature do not necessarily lead to changes in performance (Oufiero and Whitlow, 2016).

Data on single stressor effects on fish are accumulating rapidly, but knowledge on the interactive effects of multiple drivers is still scarce. There is debate as to which biotic or abiotic factors are the main drivers of macro-evolutionary change. Present water bodies already experience anthropogenic impacts, some of them (i.e. environmental chemistry) detectable by monitoring incidence of skeletal deformity. The changing climate is adding thermal, salinity and pH stress to a fragile equilibrium, and synergistic interactions are more common than additive or antagonistic interactions in marine embryos and larvae (Przeslawski *et al.*, 2015). If ecosystems commonly evolve towards fragile states, then the effect of an abiotic change may just be to trigger a collapse (Voje *et al.*, 2015).

This picture, however, infers that some elevated plasticity in phenotypes (higher variability in meristic counts and skeletal anomalies) is expected in a warming climate, at least in some species, life stages and/or populations. Future studies should elucidate the extent to which environmental variability affects the mechanics of morphogenesis that, in turn, affects phenotypic variability. More scientific understanding of critical thresholds could spur knowledge on rapid and possibly irreversible organism changes (Powell *et al.*, 2017). According to von Dassow and Davidson (2011), it is necessary to better understand the mechanical processes of tissue movement and deformation affected by variability, and in turn, the mechanics modulating phenotypic variation. Whole-organism, environment and evolutionary scales are interconnected in a framework (eco-evo-devo) where the larger scales represent the challenges that developmental mechanisms have evolved to cope.

Consequently, fundamental questions will include the effects of sublethal deformations on fish biodiversity, and if these responses will lead to permanent evolutionary change in some species.

I have reported above the functional consequences of changes to vertebral number, demonstrating that these changes are under selective predation in multiple fish species. Little is known about genetic covariation between different meristic traits in fish, while the functional significance of such covariation is poorly understood (Morris *et al.*, 2017).

We should understand if and to what extent temperature, viscosity and salinity can act as an epigenator (*sensu* Berger *et al.*, 2009), an environment signal which triggers an intracellular pathway culminating in the establishment of a stably heritable epigenetic state. Danos and Staab (2010) hypothesize that mechanical forces may have generated a novel skeletal structure possibly assimilated within the genome, and maintained regardless of environmental input.

The recent experience acquired on aquaculture-reared species showed that extreme environments will elicit epigenetic changes in phenotype; even without selection, this 'artificial' phenotype may be stabilized (genetically) through genetic assimilation *sensu* Waddington (Pfennig *et al.*, 2010). However, taking into account that various causative factors can determine the same deformities, those deformities inherited in captive conditions (e.g. saddleback in tilapia) may not necessarily be inherited in wild fish populations (Diggles, 2013).

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4

Neoplasms

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4.1 Introduction

Climate change is ongoing and it affects the aquatic environment (freshwater, marine or brackish ecosystems) by increasing water temperatures, alterations in water levels and flow regimes, eutrophication, acidification, changing nutrient loads, increasing ultraviolet (UV) light penetration, decreasing habitat and degradation, and increasing thermal stress and distribution of species (Roessig *et al.*, 2004; Marcogliese, 2008; Brierly and Kingsford, 2009; Sundh and Sundell, 2015; Frederico *et al.*, 2016; Chapman, 2018). Variations in the aquatic environment, such as temperature, salinity and chronic stress of low dissolved oxygen levels, affect mucosal barriers, the epithelia, immune cells and the internal environment (i.e. the body fluids, cells, tissues and organs) on/in aquatic organisms (Harvell *et al.*, 2002; Magnadottir, 2006; Ficke *et al.*, 2007; Sundh and Sundell, 2015; Whitney *et al.*, 2016). These lead to reduced immunocompetence in aquatic organisms, poor growth and lower reproductive performance. Most fish are ectotherms (dependent on external sources of body heat) and as such their biology, which includes metabolic and physiologic functions, is affected by water temperature and seasonality of various physiologic processes (Coffee *et al.*, 2013; Sydeman *et al.*, 2015; Whitney *et al.*, 2016).

Certain fish species, such as the anadromous salmonids, also go through major habitat changes during the parr-smolt transformation of transfer from fresh water to seawater, which are stressful events that also reduce the immune capacity of the fish (Sundh and Sundell, 2015). Climate change, which includes global warming, may also adversely affect energy reserves in fish, contributing to increased oxidative stress and decreased thermal

tolerance (Stuart-Smith *et al.*, 2015; Chapman, 2018; Reid *et al.*, 2019). In their physiological estimates of thermal sensitivity of certain ray-finned fishes (Actinopterygii), in consideration of future climate change, Comte and Olden (2017) suggested that global patterns of vulnerability to climate change differed between freshwater and marine realms. They compiled the upper thermal limits of 327 species of freshwater fish and 158 species of marine fish, and using current and future climate scenarios, predicted that marine faunas in the tropics would be most affected by climate change due to their higher intrinsic physiologic sensitivity to increased water temperatures.

4.2 Stressors and Neoplasms

Fish health has been used worldwide as an indicator of aquatic ecosystem health (Baumann, 1992; Bernet *et al.*, 1999; Frost *et al.*, 2012; Sydeman *et al.*, 2015; Blazer *et al.*, 2018), and the scientific literature on infectious diseases of fish attributed to variations in pathogen load, and increased susceptibility of fish due to climate change is abundant. Associations between environmental contaminants and the occurrence of fish neoplasms are often used as sentinels for environmental degradation (Baumann, 1992; Vijayakumar *et al.*, 2014; Singaravel *et al.*, 2017; Vergneau-Grosset *et al.*, 2017; Blazer *et al.*, 2018). Willis (1967) initially defined neoplasm as an abnormal mass of tissue whose growth exceeds, and is uncoordinated with, that of normal tissue, and which persists in the same excessive manner after removal/cessation of the initial stimulus. A more recent definition describes the cells that form the neoplasm as cells capable of division that acquire either permanent

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expressible heritable change or stable epigenetic change (Rowlatt, 1982). Neoplasms are not always discrete or restricted to a single organ, but may be diffuse within an organ, or infiltrative. The terms tumour and neoplasm are used interchangeably in the literature, though tumour often refers to any 'swelling' seen macroscopically that may represent neoplastic or non-neoplastic proliferative lesions. Occurrence of neoplasms in many fish species, for example brown bullhead (*Ameiurus nebulosus*), smallmouth bass (*Micropterus dolomieu*) and yellow perch (*Perca flavescens*), generally increases with age, and a higher prevalence of liver neoplasms has been reported in females (Martineau and Ferguson, 2006; Pinkney *et al.*, 2014, 2019). Most neoplasms are commonly found in the skin, mouth, liver and gonads.

Uncertainties still remain about direct associations between climate change and the formation of neoplasms in fish, as the cause and pathogenesis of fish neoplasms are often considered multifactorial. Groff (2004) provides an excellent review of these factors which include: (i) age; (ii) gender; (iii) genetic predisposition within and among species including hybrid fish; (iv) season/temperature; (v) presence of infectious agents (viruses, parasites); and (vi) chemical contamination of the aquatic ecosystem. For example, aflatoxins produced by the fungus *Aspergillus flavus* have been shown to induce hepatobiliary neoplasms in rainbow trout, *Oncorhynchus mykiss* (Martinaeu and Ferguson, 2006). Certain chemical compounds including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) compounds and dieldrin are cited in the literature as inducing or promoting neoplasia in fish (Baumann, 1984; Grizzle and Goodwin, 1998; Mikaelian *et al.*, 1998; Martinaeu and Ferguson, 2006; Wolf *et al.*, 2018; Pinkney *et al.*, 2019). These chemical compounds are runoffs from mining practices, agriculture sites, urban areas, oil and gas production, or waste from industrial sites. Climate change effects such as increasing water temperatures generally increase toxicity of these chemical compounds and heavy metals (Chapman, 2018), and interactions between these compounds and oncogenic viruses (e.g. retrovirus, papillomavirus and herpesvirus) are often reported to promote neoplasm development in fish (Getchell *et al.*, 1998, 2000; Coffee *et al.*, 2013).

Examples of neoplasms associated with chemical contamination of aquatic ecosystems include

oral and/or skin papillomas in brown bullhead, white suckers (*Catostomus commersonii*) and sheepshead (*Aptodinotes grunniens*), and hepatic tumours in lake whitefish (*Coregonus clupeaformis*), sauger (*Stizostedion canadense*), white suckers and walleye (*Sander vitreus*) (Getchell *et al.*, 1998, 2000; Mikaelian *et al.*, 1998; Blazer *et al.*, 2006, 2017; Pinkney *et al.*, 2014, 2019). The epidemiological study by Pinkney *et al.* (2017), however, concluded that there was no clear evidence of direct association between development of hepatic neoplasms and PCB exposure in nature given the low prevalence (< 5%) in wild smallmouth bass, *M. dolomieu*, brown bullhead, *A. nebulosus*, and juvenile yellow perch, *P. flavescens*. More recently, Pinkney *et al.* (2019) reported that polycyclic aromatic compounds initiate liver neoplasms while PCBs and DDT compounds are promoters in brown bullhead. Martineau and Ferguson (2006) noted that prevalence of neoplasms in pelagic fish (e.g. salmonids, perch) was generally low when compared with benthic species (e.g. lake whitefish, brown bullhead and white sucker), probably because of more dilute levels of environmental carcinogens such as organochlorinated compounds and heavy metals, and/or exposure to infectious agents such as retroviruses, herpesvirus and papillomaviruses.

4.3 Examples of Neoplasms

4.3.1 Skin neoplasms

In spontaneously resolving seasonal epizootics of neoplasms due to retroviruses affecting a variety of farmed and wild fish including walleye, Atlantic salmon (*Salmo salar*), white sucker, European smelt (*Osmerus eperlanus*) and Chinook salmon (*Oncorhynchus tshawytscha*) in the Great Lakes Basin, Atlantic and Pacific coasts of the USA, Canada and Europe, the largest numbers of neoplasms occurred during late spring to summer, and at spawning (Getchell *et al.*, 1998, 2000; Coffee *et al.*, 2013). Prevalence of dermal sarcomas ranged from 20% to 30% in walleye, and epidermal papillomas were at 55% in juvenile Atlantic salmon, 59% in white sucker, and 5.5% in sexually mature European smelt. Sarcomas in walleye were restricted to the skin with rare reports of invasive tumours in experimental cases.

Sweet *et al.* (2012) reported extensive melanosis and melanoma in approximately 15% of the

sampled wild population of coral trout *Plectropomus leopardus*, probably caused by environmental exposure to increased UV radiation. Fish were caught off the east coast of Australia in four sampling trips between August 2010 and February 2012. The extent of lesions in these fish varied from < 10% to almost complete skin coverage (Fig. 4.1). The fish were collected offshore in a marine protected environment with no reports of pollution, and infectious pathogens were ruled out.

4.3.2 Oral neoplasms

Spontaneous occurrence of a variety of oral neoplasms including compound and complex odontomas, odontogenic myxomas, lingual myxomas and psammomatoid ossifying fibromas in the great barracuda (*Sphyraena barracuda*) and pickhandle barracuda (*Sphyraena jello*) in marine and estuarine

environments of the south-east coast of India, had the highest seasonal prevalence (0.37–12.1% in *S. jello*) during the monsoon period, and most were found in females. The monsoon period was associated with increased water levels, and contaminated water runoff containing oil, sewage and/or industrial waste. Single or multiple masses were mainly seen in the upper jaw (Figs 4.2 and 4.3), followed by the mandible and tongue (Vijayakumar *et al.*, 2014; Singaravel *et al.*, 2017). In these fish, local-area invasion of the tongue was observed in roughly 62% of the cases, but there was no evidence of spread or metastasis into visceral organs. Using transmission electron microscopy, virus-like particles were detected in the odontomas, but definitive identification of the virus and its role in causing lesions was not established.

Gopalakrishnan *et al.* (2011) suggested the possibility that dermal neoplasms were transmitted

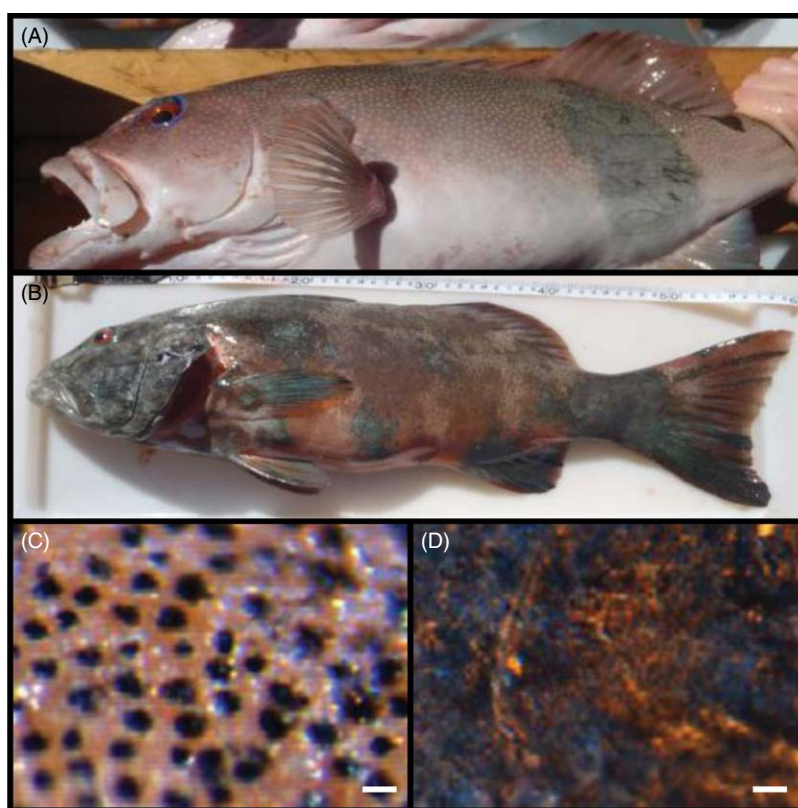


Fig. 4.1. Melanosis and melanoma in coral trout *Plectropomus leopardus*. (A) Affected fish with 10% coverage of body surface; (B) affected fish with almost complete 90% coverage; (C) microscopic examination of healthy skin; (D) microscopic examination of affected skin. Bar, 20 μ m. (From Sweet *et al.*, 2012.)

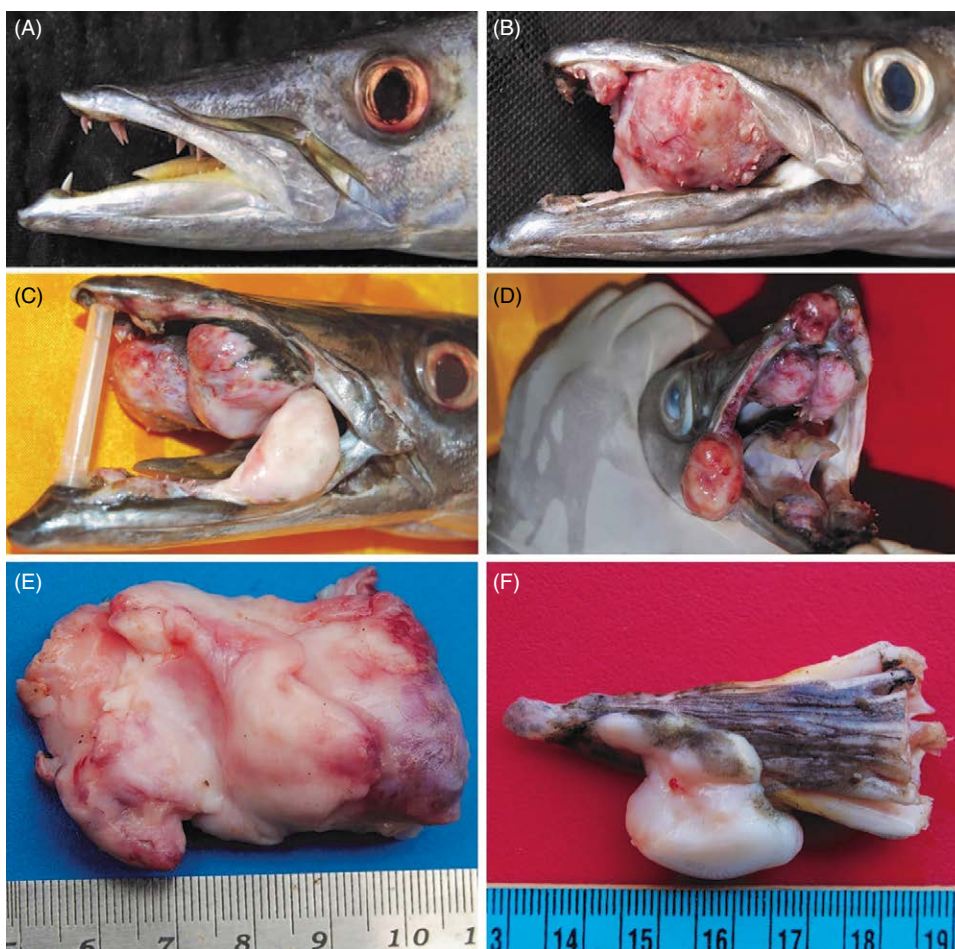


Fig. 4.2. Oral odontomas in pickhandle barracuda *Sphyræna jello*. (A) Normal fish; (B, C and D) small multinodular to large odontomas in oral cavity; (E) excised tumour mass from jaw; (F) tongue tumour. (From Vijayakumar *et al.*, 2014; copyright © 2014 Inter-Research.)

from the skin in the Indian oil sardine (*Sardinella longiceps*) to the mouth of the great barracuda. However, dermal tumours in *S. longiceps* were of mesenchymal origin, while the oral tumours in *S. barracuda* resembled odontomas, and there was no indication of an infectious aetiology in tissues examined. The sardines are a major source of prey for the barracuda, and in this instance the prevalence was considered low (0.32% in *S. longiceps* and 0.67% in *S. barracuda*), likely because both species are pelagic and live in the open ocean. Fish were collected from the south-east coast of India; the investigators did not suggest any anthropogenic factors that may have contributed to these lesions.

4.3.3 Liver neoplasms

A review article by Black and Baumann (1991) described epizootics of hepatocellular, cholangiocellular, epidermal and oral epithelial neoplasms in approximately 80 species of freshwater fishes, including commercially relevant fish such as the brown bullhead, freshwater drum (*Aplodinotus grunniens*), lake trout (*Salvelinus namaycush*), muskellunge (*Esox masquinongy*), Northern pike (*Esox lucius*), white sucker and walleye. These epizootics were mainly in adult fish (incidence ranging from 3% to 39%), and causally related to contaminant (PAHs, PCBs, chlorinated pesticides) exposure, particularly in the Great Lakes and tributary waters in the USA;



Fig. 4.3. Oral odontomas in pickhandle barracuda *S. jello*. Skeletal structure. (A) Normal supramaxilla; (B) odontomatous supramaxilla; (C) normal dentary bone; (D) odontomatous dentary bone; (E) normal premaxilla; and (F) odontomatous premaxilla. (From Vijayakumar *et al.*, 2014; copyright © 2014 Inter-Research.)

however, information on the season and gender was not included. A survey of lake whitefish from the St Lawrence River in Canada revealed alterations in hepatocytes, hepatocellular carcinoma, cholangioma and cholangiocarcinoma in fish older than 7 years attributed to the presence of chemical carcinogens, mainly PAHs, in their environment which was an indication of pollution (Mikaelian *et al.*, 1998).

Recently, Wolf *et al.* (2018) described malignant putative rodlet cell neoplasms in the livers of two, approximately 10-year-old, female white suckers, collected in late summer in the American Great Lakes region, in which sediment had been contaminated

with PAHs. Four of the remaining 98 fish collected in this study had other liver neoplasms including cholangiomas, cholangiocarcinoma and hepatocellular adenoma; total prevalence of liver neoplasms was 6%.

4.4 Conclusions with Suggestions for Future Studies

As mentioned earlier, the cause and pathogenesis of fish neoplasms are considered multifactorial involving both intrinsic factors (age, gender, reproductive state of fish) and extrinsic factors (UV radiation, oncogenic viruses, chemical contamination in the

aquatic ecosystem) among others. Neoplasms in fish rarely metastasize but can be locally invasive. The effects of climate change may vary significantly in fish, and this could be due to individual or species-specific genetics and sensitivity, population size, gender, age, position in the water column, and extent to which climate change alters the habitat in which they live. Prevalence of neoplasms in wild fish populations is generally low and in many instances associated with chemical pollution of aquatic ecosystems which is one of the most prevalent and studied consequences of climate change. As effects of climate change increase, more interactions between and among stressors that could lead to neoplasm formation are expected to increase, and thus there is expected to be more identification of neoplasms. Data on these neoplasms to date, however, are limited by random spatial and temporal fishing collection times, inconsistencies in tissue collection and methods of recording both gross and microscopic lesions in affected fish, and inadequate information on fish characteristics including gender and age. Blazer *et al.* (2018) provides necropsy-based methodology that could be used in assessment of fish populations from areas in which climate change appears to have a significant role in aquatic ecosystems.

Experiments (e.g. exposure of fish of different ages to high or low doses of contaminants at different alkalinity) to examine single stressor and interactions among multiple stressors (e.g. PAHs, PCBs, increased temperatures, low water quality, UV radiation) are needed as they may give greater insight into the cause and pathogenesis of neoplasms. Since neoplasms are generally found in adult and female fish, it would be productive to examine more closely the interactions between specific carcinogenic factors and adult fish (e.g. role of sex hormones in the development of neoplasms). The caveat though would be determining the importance of the various stressors taking into the account the inherent characteristics of the fish. Increased international cooperation in these studies across various disciplines would be beneficial as climate change is a global issue.

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5

Feeding and its Regulation

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5.1 Introduction

Climate change due to global warming results in part from the release of gases such as carbon dioxide and methane (from agricultural activities, such as livestock production; Herrero *et al.*, 2016) into the atmosphere, which trap heat radiating from the Earth (Hardy, 2003). This leads to warming of rivers and oceans, changes in precipitation and wind patterns and partial melting of ice structures, which increase sea level and affect water currents and salinities of aquatic environments (Hoegh-Guldberg and Bruno, 2010; Danladi Bello *et al.*, 2017). Increases in atmospheric CO₂ result in ocean acidification (IPCC, 2007), and decreases in overall dissolved oxygen at higher water temperatures cause hypoxic conditions (Schmidtke *et al.*, 2017). In addition, the cumulative effects of increased temperatures, increased precipitation (carrying more sediments, nutrients and pollutants) and more intense industrialization lead to eutrophication in estuarine and coastal waters and increase the severity of hypoxia (Rabalais *et al.*, 2009). These changes would lead to alterations in feeding patterns and activity of fish such as habitat ranges and migration patterns as well as on trophic food webs and interactions, and ecosystem structure and function (Rabalais *et al.*, 2009; OSPAR Commission, 2010; Scott *et al.*, 2017).

5.2 Overview of Feeding and its Regulation

5.2.1 Feeding modes, mechanisms and behaviours

Fish may be planktivores, carnivores/piscivores (fish-eating), omnivores, detritivores and herbivores (Barton, 2007). Predators may rely on several

methods to acquire food such as suction feeding or ram feeding, or a combination (Gardiner and Motta, 2012) and may hunt alone or in groups, using schooling or shoaling to optimize finding food or rounding up prey. Although relatively less energy is expended to harvest plants (herbivores, detritivores) than to capture prey (carnivores), the processing and the assimilation of plant material are long and energy costly, requiring special digestive tracts and often larger amounts of ingested food (Choat and Clements, 1998).

Food is usually detected at a distance, either visually, chemically or mechanically. At close range, some fish use visual cues to locate their food (Webster *et al.*, 2007). However, the olfactory and gustatory systems (chemoreception) are the major pathways for the detection and location of food (Carr *et al.*, 1996). The feeding behaviours of different species of fish is stimulated by different low molecular weight, water soluble chemicals, including amino acids such as glycerine, proline, ammonia and trimethylamine oxide (Carr *et al.*, 1996; Kasumyan and Døving, 2003). The acceptance (i.e. actual intake) or rejection of food also depends on chemoreception (Carr *et al.*, 1996; Lall and Tibbetts, 2009). Mechanoreception using the lateral line system can be used at close range by predator fish, as it detects disturbances created by potential prey and other moving objects (Løkkeborg *et al.*, 2014).

Electrosensory systems occur in many species including agnathans (hagfishes and lampreys), cartilaginous fish and bony fishes. Electrosensitive fish detect and orient to weak, low frequency electric fields produced by living tissues in contact with water. These signals typically indicate the presence and location of potential prey, conspecifics or predators at close range (Kempster *et al.*, 2012; Braun, 2017).

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5.2.2 Feeding rhythms

Most fish feed in distinct bouts or meals. Rhythmic feeding activity can follow diel (daily), annual (seasonal) and tidal (lunar) rhythms.

Most fish in culture systems are either diurnal (feed during the day) or nocturnal feeders (feed at night). These rhythms are generated by endogenous clock mechanisms but are also influenced by environmental factors, such as day length or temperature, which can act as synchronizers. Some fish may display both diurnal and nocturnal behavioural patterns, and shift from one phase to the other under biotic influences (e.g. increase in predation or in prey) and abiotic factors (e.g. lighting conditions). Fish may adjust their rhythms to achieve optimum feeding. For example, the marine herbivorous fish *Odax pullus* seasonally increases the proportion of protein consumed to coincide with increased energy required for the development of reproductive tissue growth (Johnson *et al.*, 2017). Annual or seasonal changes are closely correlated to environmental factors, especially water temperature and photoperiod. Fish usually increase feeding in the spring and throughout the summer when days are longer and water temperatures higher. For example, in the coral reef predator *Plectropomus leopardus*, activity patterns are directly correlated with water temperature and strike rates are highest during summer (Scott *et al.*, 2017) and in *O. pullus* seasonal foraging and reproductive behaviours of adults are governed by nutrient availability (Johnson *et al.*, 2017).

Tidal or lunar rhythms are most frequently associated with the abundance of natural prey in seawater during incoming and high tides (Madrid *et al.*, 2001; Lall and Tibbetts, 2009; Lopez-Olmeda and Sánchez-Vázquez, 2010).

5.2.3 Food intake

Generally, hunger stimulates feeding behaviour and fish feed to satisfy their energy requirements. When food is offered, fish may initially feed at a faster rate and slowly decrease or stop with a gradual decline.

Feeding depends on the quality, taste and aspect of food. Fish fed a diet low in energy may increase consumption rates to compensate for the lower caloric diet or may refuse a nutrient-deficient or unpalatable food (e.g. Boujard and Médale, 1994; Bonacic *et al.*, 2017). When given different nutrient-poor diets, fish can compose their own complete diet to meet their nutritional requirements (Sánchez-Vázquez *et al.*,

1999). Food items must be sufficiently small to be ingested, but not too large so that the fish does not waste energy eating it (Lall and Tibbetts, 2009).

Several biotic and abiotic factors may influence feeding behaviour. Biological factors include intrinsic factors such as fish size, physiological stage (nutritional, reproductive stage) and genotype, as well as social factors (subordinate versus dominant fish) and the presence of predators. Environmental factors include light, temperature, water chemical properties and water velocity (Lall and Tibbetts, 2009).

5.2.4 Intrinsic regulation of feeding

Food intake is also under close metabolic, endocrine and neuronal control, all of which are intimately related to the nutritional status of the animal.

Various endocrine and metabolic factors convey information regarding the presence of food in the gastrointestinal (GI) tract and the body's nutritional status to the fish brain, either directly or indirectly via the vagus nerve. The brain then processes this information, and neuropeptide-secreting cells are triggered to release factors that either stimulate (orexigenic factors) or inhibit (anorexigenic factors) feeding. Orexigenic factors include neuropeptide Y (NPY), orexin and agouti-related peptide (AgRP) whereas cocaine and amphetamine-regulated transcript (CART) and corticotropin releasing factor (CRF) are examples of anorexigenic factors (Volkoff, 2016; Rønnestad *et al.*, 2017).

Endocrine factors of the GI tract include cholecystokinin (CCK), ghrelin, glucagon-like peptides (GLP), insulin, amylin and leptin. With the exception of the gastric factor ghrelin, all of these factors serve as satiety signals to reduce food intake and feeding behaviour.

Certain nutrients or related compounds, such as carbohydrates, peptides, amino acids and lipids, may also affect food intake. For example, intraperitoneal injections of glucose induce hyperglycemia and cause a decrease of food intake in carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis mossambicus*) (Lall and Tibbetts, 2009; Volkoff, 2016).

5.3 Influence of Environment and Possible Effects of Climate Change

5.3.1 Climate-induced changes in the aquatic environment

Climate-induced changes in temperature and precipitation patterns affect chemical (e.g. dissolved

oxygen, salinity and nutrient concentrations) as well as mechanical properties (i.e. currents and turbidity) of aquatic environments (Whitney *et al.*, 2016) and may have profound physiological effects on fish including their feeding. These changes may also directly affect the aquatic ecosystem, and fish populations and communities by altering species distribution and diversity.

5.3.2 Effects of temperature

Most fish are ectothermic (i.e. their body temperatures closely follow that of their ambient environment). Some ocean predators (e.g. swordfish, marlins, tunas and some pelagic sharks) are endothermic, that is they have the capacity to maintain temperatures in certain tissues higher than that of surrounding water (Block, 2011). As temperature affects metabolic reactions, the ambient thermal environment tightly regulates the rate of physiological processes, and fish must occupy species-specific temperature ranges to optimize physiological performance, making fish vulnerable to climate-induced changes in temperature (Whitney *et al.*, 2016). In fish, increases in water temperatures increase cellular respiration, oxygen consumption and metabolic rates to meet aerobic energy requirements (Sandblom *et al.*, 2014). Fish species have specific temperature ranges (functional thermal tolerance windows) where specific aerobic activities (e.g. migration, digestion) are possible, and critical threshold temperatures beyond which mortality occurs (Whitney *et al.*, 2016; Pörtner *et al.*, 2017).

Temperature can affect muscles of fish, their locomotor performance and the ability to capture food or escape from predators. Muscles power the buccal cavity during the capture of prey, as well as swimming movements of a predator towards the prey, or of a prey away from a predator. Both locomotion and feeding increase with increasing temperatures up to an optimal temperature and decrease rapidly with further temperature increases (Stoner, 2004), as seen in Atlantic cod (*Gadus morhua*) (Brown *et al.*, 1989; Clark *et al.*, 1995) and channel catfish (*Ictalurus punctatus*) (Buentello *et al.*, 2000). Similarly, decreases in locomotor performance negatively affect the ability of prey to survive an attack from a predator (Higham *et al.*, 2015).

The impacts of temperature on feeding vary depending on species. In general, food intake increases with moderate temperature increases, and decreases when temperatures are outside the fish

optimal temperature range (e.g. channel catfish (Buentello *et al.*, 2000); brook trout *Salvelinus fontinalis* (Baldwin, 1957); walking catfish *Clarias batrachus* (Ahmad *et al.*, 2014); major carp *Catla catla* (Sharma *et al.*, 2017); Atlantic salmon *Salmo salar* (Breau *et al.*, 2011; Folkedal *et al.*, 2012b; Hevrøy *et al.*, 2012); clown fish *Amphiprion melanopus* (Nowicki *et al.*, 2012); common coral trout *Plectropomus leopardus* (Scott *et al.*, 2017)). However, there are also species-specific, age-specific and individual variations in the effects of temperature on feeding. For example, in two species of Minnesota, the perciform black crappie (*Pomoxis nigromaculatus*) and the catfish-like black bullhead (*Ameiurus melas*), an increase of 2°C over a period of 4 weeks results in a decrease in feeding in black bullheads but an increase for black crappies (Walberg, 2011). In juvenile Atlantic salmon exposed to elevated temperatures, 1- and 2-year-old fish cease feeding whereas younger fish continue foraging (Breau *et al.*, 2011). In brown trout (*Salmo trutta*) raised under similar conditions at a high but sublethal temperature, some individuals maintain food intake to support growth, whereas others eat little and lose weight, which is correlated to individual differences in mitochondrial capacities of both their liver and their white muscle (Salin *et al.*, 2016).

Temperature also affects taste preferences, some fish displaying different preferences for the same food items in warm and cold waters (Kasumyan and Døving, 2003), and feeding periodicity (Fraser *et al.*, 1993).

Few studies have examined the role of endocrine factors in these changes. In Atlantic salmon, temperature-induced variations in feeding have been correlated to changes in plasma concentrations of ghrelin (Hevrøy *et al.*, 2012; Vikeså *et al.*, 2017) and leptin (Kullgren *et al.*, 2013). In Atlantic cod, low temperatures inhibit food intake, and this inhibition is in part mediated by increases in CART expression (Kehoe and Volkoff, 2008). In cunner (*Tautoglabrus adspersus*), orexin, NPY, CART and CCK mRNA levels decrease during natural torpor (when fish are exposed to low temperatures and do not feed) as compared with fed summer fish, suggesting these hormones mediate different physiological responses to torpor-induced long-term fasting (Babichuk and Volkoff, 2013).

The hypothalamic–pituitary–interrenal (HPI) axis is also involved in temperature-mediated changes in feeding. Stressful environmental conditions such as

increase in temperature induce a neuroendocrine stress response and the release of CRF and cortisol which are anorexigenic factors in fish (Bernier, 2006; Madison *et al.*, 2015). Extreme high and low water temperatures induce an increase in cortisol levels in several species (e.g. grouper *Epinephelus akaara* (Park *et al.*, 2016); salmon (Strange *et al.*, 1977; Folkedal *et al.*, 2012a); carp (Jaxion-Harm and Ladich, 2014)).

Temperature also affects the fish immune system (see Chapter 11, this volume), the function of which is most efficient within the optimal temperature range of a species (Ellis, 2001; Dittmar *et al.*, 2014). Temperatures deviating from that optimum can decrease immune function, through the release of cortisol (Whitney *et al.*, 2016). Since diseased fish and fish with weak immune systems stop feeding (Volkoff and Peter, 2004), variations in temperature can have indirect effects on feeding behaviour.

Temperature-induced reductions in feeding rates are associated with reduced conversion efficiency of ingested food (Jobling, 1997; Nowicki *et al.*, 2012). Digestive processes and nutrient/energy digestibility usually decrease at temperatures outside the optimal range (e.g. brook trout; Amin *et al.*, 2016). These changes are due in part to the effect of temperature on digestive enzyme activities. In walking catfish, total protease, trypsin and chymotrypsin activities are higher in fish exposed at 25°C compared with lower temperatures, lowest enzyme activities being recorded at 10°C (Ahmad *et al.*, 2014). In major carp, fish exposed at 10°C have reduced digestive enzymes (amylase, protease, lipase, trypsin) activities (Sharma *et al.*, 2017) compared with fish held at 25°C. Similarly, in cunner, low temperatures induce a reduction in the activities of trypsin, alkaline phosphatase and lipase, and a decrease in mRNA levels of alanine aminopeptidase (Hayes and Volkoff, 2014). Standard dynamic action (SDA, the increase in energy expenditure during ingestion, digestion, absorption and assimilation of a meal) is also affected in some fish held at extreme temperatures (e.g. cod; Tirsgaard *et al.*, 2015b).

At the population level, behavioural and physiological changes induced by changes in temperature could ultimately affect predator–prey dynamics within fish communities (Domenici *et al.*, 2007a). For example, the large predatory brown trout cannot sustain high metabolic costs required at higher temperatures which might lead to starvation and disappearance from the population, thus benefiting its main prey species, the smaller Arctic charr

(*Salvelinus alpinus*) (Abrahams *et al.*, 2007). Similarly, as a consequence of global warming, tropical invasive species are expected to expand their range to cooler waters, which might have negative impacts on ecosystems (Turingan and Sloan, 2016). Changes in the species composition and feeding ecology might also be the result of changes in migration patterns in several species, such as Atlantic cod (Kell *et al.*, 2005; Perry *et al.*, 2005), Atlantic mackerel (*Scomber scombrus*) and European flounder (*Platichthys flesus*) (Sims *et al.*, 2004).

5.3.3 Effects of salinity

Changes in salinity affect food intake in both marine and freshwater fish. For example, in marine yellowtail kingfish (*Seriola lalandi*), higher growth rates and increased feed intake occur at lower salinities as opposed to high salinities (Blanco Garcia *et al.*, 2015), and in sub-Antarctic notothenioid (*Eleginops maclovinus*) (Vanella *et al.*, 2017) and grey mullet (*Mugil cephalus*) (De Silva and Perera, 1976; Barman *et al.*, 2005), high and low salinities decrease both food intake and growth. Within freshwater fish, high salinities increase mortality, and decrease locomotor activity, feeding and growth and increase cortisol levels in goldfish (Luz *et al.*, 2008), tambaqui (*Colossoma macropomum*) (Fiúza *et al.*, 2015), and the catfish (*Lophiosilurus alexandri*) (Mattioli *et al.*, 2017) and *Pangasianodon hypophthalmus* (Nguyen *et al.*, 2014). In the euryhaline (fish which live in habitats such as estuaries and tide pools) European sea bass (*Dicentrarchus labrax*), lowering the salinity levels reduces food intake (Rubio *et al.*, 2005). Similarly, in the euryhaline pejerrey (*Odontesthes bonariensis*), high salinities induce the upregulation of nesfatin-1, an anorexigenic peptide, suggesting an anorexigenic effect of the increase in water salinity (Bertucci *et al.*, 2017). However, in estuarine black bream (*Acanthopagrus butcheri*), salinity does not appear to affect food intake but negatively affects growth (Partridge and Jenkins, 2002). In some fish (e.g. Nile tilapia (Tran-Ngoc *et al.*, 2017) and glass eel (*Anguilla anguilla*) (Ciccotti *et al.*, 1993)), although changes in salinity do not appear to affect food intake, they affect intestinal morphology and digestive processes which suggests they might have negative effects on digestion and assimilation of food.

Salinity can also affect sensory systems. For example, in glass eels, chemotaxis in response to some organic odorants can switch from negative or

positive, depending on the environmental salinity (Sola and Tongiorgi, 1996). In stingray (*Dasyatis sabina*), the sensitivity and detection range of the electrosensory system is reduced in lower salinity water (McGowan and Kajiura, 2009).

Changes in salinities can also have impacts on ecosystems and trophic levels. For example, in seas that are highly influenced by river runoff, such as the Baltic, Mediterranean and Black Sea, increases in fresh water due to enhanced rainfall could lead to a shift from marine to more brackish and even freshwater species, thus changing the natural dynamics between predators and prey (Philippart *et al.*, 2011; Torossian *et al.*, 2016).

5.3.4 Effects of dissolved gases and pH

Ocean acidification and elevated CO₂ levels can negatively affect fish biology, altering physiology and behaviours as well as modifying ecosystem function. Increases in CO₂ levels may lead to hypercapnia (elevated CO₂ partial pressure, pCO₂) and acidosis in the blood and tissues of fish, inducing changes in metabolic function and growth (Pörtner *et al.*, 2004; Potts *et al.*, 2015). The biological consequences of ocean acidification and elevated pCO₂ may have major impacts on marine food webs, nutrient cycles and overall productivity (Hasler *et al.*, 2016).

Changes in dissolved CO₂ and water pH reduce food intake in several bony fish, including sea bass (Cecchini *et al.*, 2001). Food intake is also reduced by hypoxia in a dose–response-like pattern in several species, including salmon (Remen *et al.*, 2012; Vikeså *et al.*, 2017), trout (Glencross, 2009) and Atlantic cod (Thorarensen *et al.*, 2017). In the filter-feeding silver carp (*Hypophthalmichthys molitrix*), filtration rates and filtering efficiency decrease at low oxygen levels (Zhao *et al.*, 2017). This decreased feeding results in decreases of growth rates, as in pink salmon (*Oncorhynchus gobuscha*) (Ou *et al.*, 2015; Hasler *et al.*, 2016) and Atlantic salmon (*S. salar*) (Vikeså *et al.*, 2017). Exposure to elevated CO₂ can also impair feeding behaviours and activities of sharks, as in Port Jackson sharks (*Heterodontus portusjacksoni*) reared under elevated CO₂ conditions (Pistevos *et al.*, 2017). High CO₂ levels reduce attraction to food and attack behaviour in smooth dogfish (*Mustelus canis*) (Dixson *et al.*, 2015), and result in fewer swimming events in lesser-spotted catsharks (*Scyliorhinus canicula*) (Rosa *et al.*, 2017). In addition, low pH

water has been shown to shift taste preferences, perhaps by modifying palatability of different foods (Kasumyan and Døving, 2003).

Small changes in levels of dissolved gases and pH can also affect sensory systems and anti-predator responses in fish (Leduc *et al.*, 2013). Anoxic conditions impair visual function in fish and may result in decreases in feeding and escape behaviours (Domenici *et al.*, 2007b; McCormick and Levin, 2017). Behavioural responses to basic chemical stimuli from food (amino acids) are reduced at low pH in predators such as fathead minnows (*Pimephales promelas*) (Dennis Lemly and Smith, 1985), Atlantic salmon (Royce-Malmgren and Watson, 1987) and pink salmon (Ou *et al.*, 2015). These changes also affect elasmobranchs, as high CO₂-treated sharks avoid food-related odour cues and reduce their attack behaviour (Dixson *et al.*, 2015). The disruption of the ability to detect and respond to chemical cues under acidified conditions also makes prey fish more prone to predation. Exposure to alarm cues normally elicits predator avoidance behaviour (e.g. reduced activity levels, increased hiding or formation of tighter groups). This behaviour is diminished or absent when alarm/conspecific cues occur in waters with low pH and/or high CO₂ conditions (e.g. minnow (Dennis Lemly and Smith, 1985; Tix *et al.*, 2017); pink salmon (Ou *et al.*, 2015); Atlantic salmon (Leduc *et al.*, 2009); brook trout (*Salvelinus fontinalis*) (Leduc *et al.*, 2013)). It has been suggested that the behavioural changes observed in teleosts at high CO₂ might be caused in part by modifications of the γ -aminobutyric acid (GABA)-A receptor, the primary inhibitory neurotransmitter receptor in the vertebrate brain (Lai *et al.*, 2015; Vossen *et al.*, 2016). Overall, failure to appropriately respond to chemical cues could change feeding patterns and increase mortality rates of prey fish, thus potentially altering food web dynamics.

Species-specific differences in response have been shown, as some fish are more resilient to changes in CO₂ and pH (Heuer and Grosell, 2014). For example, high CO₂ levels do not alter the behaviour of either the benthic reef-dwelling shark (*Hemiscyllium ocellatum*) (Heinrich *et al.*, 2016), silver carp (*Hypophthalmichthys molitrix*) (Tix *et al.*, 2017), Atlantic cod (Jutfelt and Hedgärde, 2013) or goldsinny wrasse (*Ctenolabrus rupestris*) (Sundin and Jutfelt, 2016). These species-specific differences may be due in part to an adaptation to low environmental oxygen conditions and normal diel

fluctuations in CO₂ encountered in some fish habitats (Heinrich *et al.*, 2016).

Interactions of ocean warming and acidification have been shown in several fish. For example, in clown fish (*Amphiprion melanopus*), CO₂ levels alone do not affect food consumption and foraging activity, but rearing at high temperature and high CO₂ results in an increase in feeding behaviour, suggesting an interaction between CO₂ levels and temperature (Nowicki *et al.*, 2012). In sharks, although high CO₂ levels alone have negligible effects, the combination of high temperature and high CO₂ reduces the potential of fish to locate prey (Pistevos *et al.*, 2017).

High CO₂ levels also affect digestive processes. In Atlantic cod, exposure to high CO₂ levels prolongs the time for digestion and SDA, and decreases digestive efficiency (Tirsgaard *et al.*, 2015a). In toadfish (*Opsanus beta*), CO₂ exposure causes an increase of intestinal HCO₃⁻ secretion, which might disrupt the intestinal milieu associated with digesting a meal, and thus affect digestive functions (Heuer and Grosell, 2016).

5.3.5 Effects of water currents, turbidity and light levels

Most fish live in habitats with movement of water, such as rivers, open oceans, coral reefs and coastlines. This movement creates specific current and turbidity levels, which can affect the locomotion and feeding of fish. Water movements can affect the survival of fish, as some fish use high speed swimming in fast flowing water (e.g. tuna, salmon), whereas others (e.g. carp, pufferfish) live in relatively still waters and cannot handle high currents. Climate change modifies precipitation and wind patterns, with an increasing frequency and intensity of storms, leading to higher winds and larger tides and currents (Johansen, 2014; Johansen *et al.*, 2015b). Global warming may also modify wave patterns through increases in the number of ship days and in the frequency of boating in cold/temperate zones (Shaw and Loomis, 2008; Gabel *et al.*, 2017). Changes in waves/turbulence can potentially affect foraging and locomotion in fish.

Capturing prey and escaping from predators rely on accurate motor control and an acute sensory system. Turbulent waters can negatively affect fish by reducing swimming speed capabilities, and increasing oxygen consumption and energy expenditure needed to sustain swimming efforts to catch

prey (Liao and Cotel, 2013). For example, in Pacific bluefin tuna (*Thunnus orientalis*), feeding rates decrease at both higher and lower turbulence levels (Kato *et al.*, 2008), and in larval Atlantic cod, prey hunting success decreases as turbulence increases (MacKenzie and Kiørboe, 2000). Rapidly flowing or turbulent water may also compromise the ability of a prey fish to detect a predator as many prey fish utilize their lateral line system, which is sensitive to flow and water disturbances, to detect approaching predators (Higham *et al.*, 2015). Changes in water flows may lead to changes in habitats and ecosystems, leading to major changes in the distribution and diversity of fish species thus possibly resulting in imbalances between prey and predators (Higham *et al.*, 2015).

Increases in turbulence as well as changing rainfall patterns (which increase the amounts of nutrients, sediment and detritus brought by rivers into the coastal zone) may increase turbidity, creating low visibility, and thus influencing feeding in fish (Potts *et al.*, 2015). In some cases, increased currents may have beneficial effects as more benthic prey organisms are suspended in the water and more accessible to feeding fish (Gabel *et al.*, 2011). However, turbidity reduces levels of light and the visual range of fish, and can affect their predator and prey detection. The influence of changing ambient light is species specific (Higham *et al.*, 2015). Some predatory fish are diurnal feeders, whereas others feed in low light conditions (at dawn and dusk or at night). Predators feeding in the dark usually use olfaction and the lateral line, whereas diurnal predators usually use vision to detect prey (Higham *et al.*, 2015). It has been suggested that the preference of predators to feed in low light is in part due to a compromised visual system of prey (e.g. minnows; Pitcher and Turner, 1986) and the reduced frequency of anti-predator behaviours (e.g. schooling) as light decreases (e.g. blacknose dace (*Rhinichthys atratulus*) preyed by creek chub (*Semotilus atromaculatus*)) (Cerri, 1983). Visual predators are the most affected, and their feeding rates decline with increasing turbidity (Suursaar *et al.*, 2015) (e.g. piscivorous sablefish (*Anoplopoma fimbria*) (De Robertis *et al.*, 2003), the insectivorous fish *Moenkhausia forestii* (Figueiredo *et al.*, 2016) and planktivorous coral reef damselfishes (Pomacentridae) (Johansen and Jones, 2013)). Some species, such as the largemouth bass (*Micropterus salmoides*), can change their predatory strategy, switching from visual cues in the light to olfactory

and mechanical stimuli in darkness (Gardiner and Motta, 2012), which probably makes them less vulnerable to fluctuations in turbidity.

Changes in turbidity also may affect ecosystems, where better-adapted non-visual fish species may become dominant. For example, whereas both species perform well in low turbidity waters, the New Zealand invasive Western mosquitofish (*Gambusia affinis*) has a better ability to forage under high turbidity levels than the native inanga (*Galaxias maculatus*), which exhibits a decline in feeding rates (Abrahams *et al.*, 2017).

In the Arctic Ocean as well as in Alpine and high latitude lakes, the decline in the ice cover results in more light penetrating waters. This may increase vision-based foraging of planktivorous fish but also increases predation risks, with potential effects on trophic systems and ecosystems (Langbehn and Varpe, 2017).

5.3.6 Effects of contaminants

Intensifying anthropogenic land use and climate change (increased rainfall and melting snow and ice) lead to an increase in the flow of organic matter and contaminants into aquatic ecosystems (Jonsson *et al.*, 2017). Contaminants can accumulate in plankton and small invertebrates at the base of the trophic web and become more concentrated in larger fish and affect feeding.

For example, many pollutants, such as oil, pesticides, heavy metals or detergents affect fish taste reception by both destroying the taste buds and reducing the sensitivity to the taste stimuli, thus impairing their ability to feed (Kasumyan and Døving, 2003; Gardiner *et al.*, 2017). Mercury can act as a neuroendocrine disruptor, as seen in methyl-mercury-treated largemouth bass in which neuropeptide receptor and steroid signalling are altered (Richter *et al.*, 2014). In rare minnow (*Gobiocypris rarus*) larvae, mercury (Hg) exposure disrupts the stress axis and the thyroid axis (Li *et al.*, 2014), both of which affect feeding in fish (Volkoff, 2016). The lateral line system can be damaged by antibiotics (e.g. zebrafish (*Danio rerio*); Owens *et al.*, 2009), toxic chemicals (such as bleach, e.g. zebrafish; Amorim *et al.*, 2017) or heavy metals (e.g. zebrafish (Hernández *et al.*, 2006); sea bass (Faucher *et al.*, 2008)), potentially affecting their locomotion and ability to detect prey or escape predators. In addition, in some fish (e.g. ornate wrasse *Thalassoma pavo*), toxins such as

pesticides (Zizza *et al.*, 2017) and lead (Zizza *et al.*, 2014) increase the brain expression of orexin receptors and impair swimming and motor performances and feeding behaviour.

5.4 Conclusions and Future Research

The vulnerability of individual species and populations to climate change is influenced by their ability to develop short-term changes in behaviour and physiology (acclimatization) and long-term generational genetic changes (adaptation). Consequently, inter-species differences in sensitivity to climate change are broad and depend on species-specific physiological characteristics and adaptations to their habitats. Resident tropical species living near the equator and exposed to limited diurnal and seasonal variations in temperature, are predicted to be more vulnerable to increasing temperatures than temperate species, which live in waters where temperatures vary cyclically (Sunday *et al.*, 2011; Potts *et al.*, 2015; Sandblom *et al.*, 2016; Habary *et al.*, 2017; Scott *et al.*, 2017).

Some fish exhibit behavioural plasticity and alter their foraging/feeding behaviour and activity as temperatures change to compensate for increased energetic needs, either through increased food intake and/or reduced energy investment (Johansen *et al.*, 2014, 2015a). For example, lake trout (*Salvelinus namaycush*) adjust their feeding behaviour annually according to water temperature by eating more large prey from shallow near-shore regions in cooler years and more small prey from deep offshore regions (Guzzo *et al.*, 2017). Fish may also increase their time spent resting to regulate increasing metabolic costs at higher temperatures (Habary *et al.*, 2017). However, changes in behavioural patterns may affect not only individual fitness, but also species interactions (predation and predator evasion), food webs and thus biodiversity in ecosystems (Scott *et al.*, 2017). Furthermore, to avoid warming waters, some poorly adapted migratory species may move to more favourable temperatures and thus alter their distributional range and disrupt food webs (Feary *et al.*, 2014; Scott *et al.*, 2017).

A few bony fish switch feeding modalities when deprived of some of their senses, allowing them to feed on more diverse prey in a wider variety of environments. For example, in largemouth bass, visual deprivation induces a switch from visual predation to suction-based feeding and lateral line

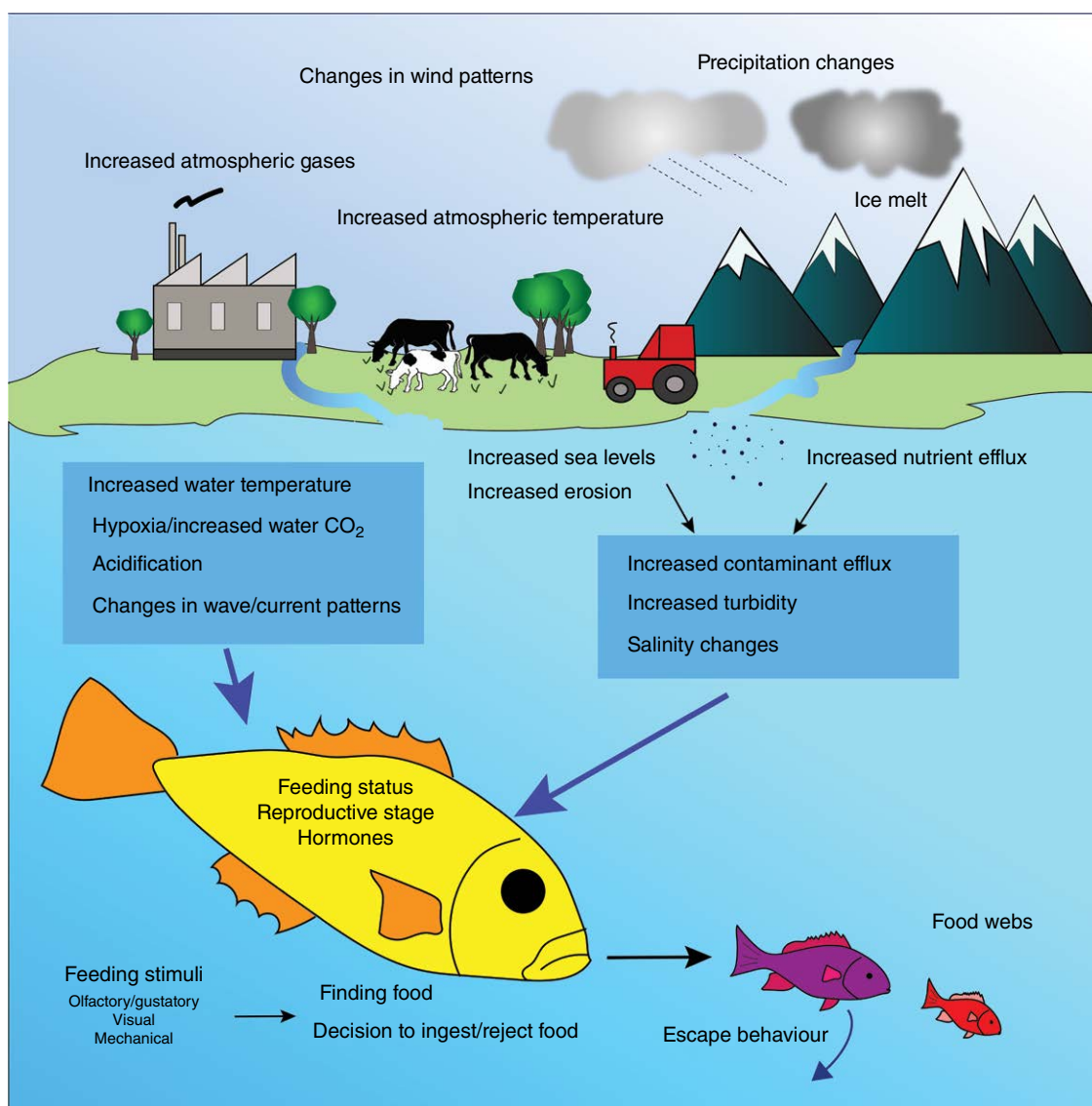


Fig. 5.1. Schematic view of possible effects of climate change on feeding in fish (e.g. marine teleosts). Feeding in fish involves the search for food and the actual ingestion, or rejection, of food, both being dependent on feeding stimuli received by the sensory system. Prey fish also use senses to initiate escape behaviour. Feeding behaviour is regulated by intrinsic factors (e.g. appetite-regulating hormone levels, reproductive stage and feeding/hunger status) but is also influenced by environmental factors (e.g. temperature, photoperiod). Climate change results, in part, from the release of gases into the atmosphere, which leads to warming of rivers and oceans, changes in precipitation and wind patterns and melting of ice, increasing the sea level and affecting currents and salinities of aquatic environments. All these changes have been shown to have physiological effects on some fish species and affect their feeding behaviour, which in turn might affect food webs, population dynamics and ecosystems. (Original figure designed by H. Volkoff.)

deprivation results in higher forward velocities during capture, leading to a better success in prey capture (Gardiner and Motta, 2012). Elasmobranchs appear to be less plastic than teleosts, as they do not switch feeding modalities in response to sensory deprivation, despite slight modifications of their prey capture behaviour, which include angle of attack or elevation of head (Gardiner *et al.*, 2017).

Furthermore, responses to climate change may vary within species, some fish coping better under warming conditions than others. Responses may differ between males and females and between individuals at different life stages (Whitney *et al.*, 2016) as well as among individuals at comparable physiological stages (Salin *et al.*, 2016).

In on-land aquaculture (currently used to maintain broodstocks and larvae, which are more sensitive to water quality), the environment can be relatively easy to control by modifying and maintaining water parameters, such as temperature, dissolved oxygen/CO₂ levels and salinity. However, in open ocean cages, the control of ambient conditions such as ocean currents and temperature is problematical (Klinger *et al.*, 2017). In addition, the effects of climate change will be likely to vary depending on the geographical location. Aquaculturists may have to move their operations to more favourable environments (perhaps away from the equator and towards the poles), but this may be costly, logistically difficult and politically challenging (Klinger *et al.*, 2017; Lazard, 2017). Another option is to switch to species more adapted to new environmental conditions, or use selective breeding or develop genetically modified fish to increase genotype-related feeding and growth (Dahm and Geisler, 2006; Chen *et al.*, 2015), all of which may require long-lasting and costly new expertise and infrastructures.

The potential effects of climate change on fish feeding behaviour and food intake, resulting in potential alterations in food webs are summarized in Fig. 5.1. These effects cannot be generalized, as they may change according to several parameters, such as species-specific physiology and behaviour, as well as geographical location of habitats. Thus far, relatively few studies have been carried out on a limited number of species and these use different climate models (Freer *et al.*, 2018). Furthermore, most studies to date expose experimental fish to very rapid and extreme environmental changes and these are likely to be more extreme than those faced by wild populations of fish. Consequently, our current models may perhaps overestimate the

impacts of climate change on fish (Scott *et al.*, 2017). Future studies should examine multiple fish species exposed to more gradual changes and diverse environmental variations under both experimental and natural conditions to produce standardized models which may help us better understand the effects of climate change on feeding in fish.

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6

Nutritional and Metabolic Disorders

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6.1 Introduction

Environmental climate change includes fluctuations in temperature, salinity, aqueous gases and eutrophication. In general, this means waters and local sea areas that are warmer, more acidic, lower in oxygen levels, higher in carbon dioxide concentrations and more polluted. For farmed fish species housed in restricted areas and water volumes, such changes in environmental conditions are met by individual adaptation in behaviour, nutrition and overall physiology. Severe long-lasting environmental conditions or more frequently occurring extreme episodes affect fish growth performance and welfare, as well as increasing local environmental burdens (e.g. eutrophication caused by wasted food due to lower feed conversion efficiencies). How wild and farmed fish handle these changes has been studied in detail, with the aim to optimize conditions for farmed fish performance and health through the production cycle. The present chapter aims to collate research on how climate change, in farming conditions, may affect nutrition and metabolism and related production disorders in fish. Examples will focus on salmonids, as a major farmed species, in both freshwater and marine environments.

6.2 Major Climate Change for Aquaculture

A report from the Intergovernmental Panel on Climate Change (IPCC) (2013) shows that the global average land and ocean surface temperature has increased by 0.85°C since 1880, and the temperature is predicted to continue to increase. The main scenario for climate change in northern Europe and North America in the near future will be milder, wetter and stormier winters. How will these changes

affect aquaculture and fish welfare? For aquaculture there will be greater impacts in fjords and local coastal areas than in the open ocean (Bergh *et al.*, 2007). For example, it may be expected that there will be periods when temperatures are above the optimum for fish growth, and both temperature fluctuations and the longer duration of periods when these occur will present a continuous challenge for farming along the Norwegian coast. Along with temperature fluctuations, corresponding changes in dissolved gases and eutrophication from suboptimal feed utilization will be experienced. Since availability of oxygen is one of the most critical water quality parameters in aquaculture, hypoxia at elevated temperatures represents additional metabolic stress for farmed fish with reduced growth and performance as a result (Wang *et al.*, 2009).

The water temperature normally changes with the seasons during a commercial farming production cycle in temperate climate zones (Waagbø *et al.*, 2013). There is a risk of high losses in production of Atlantic salmon (*Salmo salar*) in southern to western Norway during the summer in periods of extreme elevated sea temperatures or temperature fluctuations. Temperatures above the optimum for growth of farmed salmonids may result in reduced appetite, growth, feed efficiency and immunity, and increased risk of production-related disorders and unspecific mortalities (Hevrøy *et al.*, 2012, 2015; Vikeså, 2017). Secondary effects of thermal stress may be linked to reduced immune capacity and the increased risk of infectious diseases (see Chapter 11, this volume). Basic knowledge of how thermal stresses affect fish will be very important for future sustainable fish farming. The question arises at which organizational level, molecular, cellular or organismic, the limits of thermal tolerance are likely, and if there are measures to increase the robustness of farmed fish to cope with these challenges.

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6.2.1 Temperature tolerance in fish

Temperature tolerance and the length of time for an effect to arise differ by level of biological organization (e.g. molecules, organelles, cells, tissues, organism and population) as well as the adaptive mechanisms responsible for long- and short-term tolerance (Pörtner, 2002). The thermal limits for salmonids are species specific: for example Atlantic salmon has the highest temperature tolerance, followed by brown trout (*Salmo trutta*), and Arctic charr (*Salvelinus alpinus*) has the lowest. In general, the temperature tolerance for parr and smolts are higher than for alevins and developing eggs and for adults and broodstocks. The upper limits for feeding are also highest for Atlantic salmon and lowest for charr, while the lower limits are close to zero for all three species. When water temperature extremes exceed 22–28°C, Atlantic salmon will die if they are not moved to cooler water (Elliott and Elliott, 2010). Fish used in aquaculture in seawater are kept in net pens and do not have the same ability to escape from unfavourable environmental conditions as fish in the wild.

6.3 Nutrition and Metabolic Responses to Climate Change

6.3.1 Temperature – feed intake, growth and feed utilization

Research related to the metabolic and welfare implications of climate change are mostly done in controlled environments and with salmon or other species relevant to commercial aquaculture production. The effects of temperature are the most studied in fish species. Metabolism and growth are influenced by water temperature, in addition to nutritional requirements and nutrient availability, uptake and utilization (Jobling, 1994). When feeding *ad libitum*, the growth rate will increase up to the optimum temperature for growth and drop at higher or lower temperatures. The optimum temperature for growth may differ for different size classes of fish species. For instance, the optimum temperature for Atlantic salmon post-smolts is in the range of 12.8°C (70–150 g) to 14.0°C (150–300 g) (Handeland *et al.*, 2008). The optimum temperature for adult Atlantic salmon (above 1.6 kg) appears to be in the same range, as Hevrøy *et al.* (2013) found a higher growth rate in salmon reared at 13°C compared with salmon reared at 15°C, 17°C and 19°C. Only minor differences were found for 1 g, 50 g and 300 g

body sizes of brown trout, with the mean optimal temperature range of 13.6–14.1°C (Elliott and Hurley, 1999). For Atlantic halibut (*Hippoglossus hippoglossus*), the growth rate was higher at lower temperatures for large fish (Björnsson and Tryggvadóttir, 1996). Similarly, the optimal temperature for growth decreased with increasing fish size for juvenile spotted wolf fish (*Anarhichas minor*) (Imsland *et al.*, 2006) and for juvenile lumpfish (*Cyclopterus lumpus*) (Nytro *et al.*, 2014).

The basal metabolism of fish increases with increasing temperature, and at high temperatures the elevated energy requirement for maintenance results in less energy available for growth (Jobling, 1994). This may be evident through lower feed efficiency and retention of nutrients, often accompanied by reduced feed intake at high temperature. Elliott and Hurley (1999) predicted positive energy change (growth) and energy loss in brown trout reared at 3.6–20.4°C, in relation to ration size (daily energy intake). The model showed that at a maximum ration level brown trout at 18°C would have equal values for energy intake and energy losses. The optimum temperature for growth varies little among populations within a species, whereas the growth efficiency may be locally adapted to the temperature conditions. Since the optimal temperature also decreases with decreasing energy intake, the ration level should always be given when defining an optimal temperature for growth (Jonsson and Jonsson, 2009).

Effects of temperatures above the optimum on feed intake and growth are dependent on exposure time and size of fish. Several studies have shown reduced feed intake and feed efficiency at high temperature, as well as differences between optimum temperature for growth and feed efficiency between different size classes. Examples include juvenile spotted wolf fish (Imsland *et al.*, 2006), Atlantic salmon (Handeland *et al.*, 2008) and Atlantic halibut (Björnsson and Tryggvadóttir, 1996).

High temperatures also influence retention of energy and nutrients. In regression studies on two sizes (21 g and 142 g) of barramundi (*Lates calcarifer*) exposed to 23–38°C, reduced growth, feed intake, feed efficiency and protein retention were seen at the highest temperature (Bermudes *et al.*, 2010). In that study, the maximum feed intake was found in wider temperature ranges for small fish (29.1–34.9°C) than for large fish (32–34.9°C). In large (1.6 kg) Atlantic salmon reared at 19°C versus 14°C, reduced feed intake, feed conversion and

growth was found in fish reared at high temperature (Hevrøy *et al.*, 2012). In this study, both somatic indexes, as well as retention of protein and lipid were significantly reduced, indicating an increased use of endogenous resources resulting in energy depletion. In a study on Atlantic salmon (175 g) reared at 8°C, 12°C or 18°C for 3 months, feed intake, growth and condition factor were similar between all groups after 1 month, but declined in the fish exposed to 18°C after 3 months (Kullgren *et al.*, 2013). In adult Atlantic salmon (1.6 kg) subjected to temperatures of 13°C, 15°C, 17°C or 19°C for 45 days, the daily feed intake did not differ during the first 15 days, however, during the last 30 days the feed intake decreased with increasing temperature. Growth was reduced in the fish exposed to 19°C already after 15 days, but after 45 days growth was significantly reduced with increasing temperatures and the feed efficiency declined. Retentions of protein, lipid and energy were significantly reduced at 19°C, where the decline in lipid retention was most prominent (Hevrøy *et al.*, 2013). In a comparative study between rainbow trout (*Oncorhynchus mykiss*) (125 g) and Atlantic salmon (100 g) post-smolts reared at 13°C or 19°C for 35 days, no differences were seen in feed intake or weight gain, while length growth was reduced in both Atlantic salmon and rainbow trout reared at elevated temperature (Hevrøy *et al.*, 2015). In yellowtail kingfish (*Seriola lalandi*), no differences were seen in feed intake, despite lower growth rate at 26°C than in 21°C (Ilham and Fotedar, 2016).

The reduction in feed efficiency results in economic losses for fish farmers, as well as increased environmental burdens due to increased waste outputs. Waste output, feed conversion and growth may also be influenced by digestibility of the feed. In a study of Atlantic salmon (3.4 kg), the digestibility of protein and fat was not changed at temperatures between 6°C and 11°C (Ng *et al.*, 2004). In different size classes of rainbow trout reared at 7°C, 11°C and 15°C, digestibility of protein, lipid and energy was lower for 18 g fish compared with 207 g and 586 g fish reared at 7°C, while no differences were detected at higher temperatures (Windell *et al.*, 1978). The digestibility of saturated fatty acids was lower in 45 g rainbow trout reared at 7°C compared with 15°C (Ng *et al.*, 2003). In barramundi, a temperature-dependent effect (23–38°C) was seen with increased digestibility of energy, dry matter and phosphorus at high temperatures, and a higher waste output at the lowest temperature

(Bermudes *et al.*, 2010). Similarly, Kullgren *et al.* (2013) found a higher protein digestibility in Atlantic salmon reared at 18°C compared with 12°C and 8°C. Digestion requires oxygen, which may be insufficient at high temperature, however, how digestion is affected by even higher water temperatures is not known.

Growth is regulated by a complex array of endocrine processes, including the growth hormone (GH)–insulin-like growth factor (IGF) system. Briefly, the production of the anabolic hormone IGF-1 is dependent on GH production and release from the pituitary, which is affected by the environment, endogenous rhythms and negative feedback control by GH and IGF-1 (Björnsson *et al.*, 2002). GH binds to the growth hormone receptor (GHR) that stimulates production of IGF-1 in the liver, increases the level of circulating IGF-1, and also acts directly on target tissues to stimulate autocrine/paracrine actions of IGF-1 (Björnsson *et al.*, 2002). In several studies plasma concentrations of IGF-1 have been shown to be positively correlated with the growth rate, although some have found no correlation, possibly due to different physiological conditions and environmental factors, including rapid temperature changes (reviewed by Beckman, 2011). IGF-1 production is also influenced by the nutritional status, feed intake (appetite), feed ration, metabolism and nutrient assimilation. The availability of IGF-1 to bind to receptors in target tissues is regulated by IGF-binding proteins (IGFBPs), where IGFBP-1 is a negative regulator that slows down growth when the physiological state is catabolic (Kawaguchi *et al.*, 2013).

Reduced growth and feed intake in Atlantic salmon reared at 19°C compared with 14°C was linked to reduced circulating ghrelin (reduced appetite) at high temperature, resulting in a negative energy status and downregulation of appetite through defined endocrine factors in the hypothalamus such as neuropeptide Y (NPY), pro-opiomelanocortin A2 (POMC A2) and cholecystokinin-L (CCK-L), indicating an anorexic state (Hevrøy *et al.*, 2012). In Hevrøy *et al.* (2013), an increased expression level of *ghr* was found in muscle of salmon reared at 19°C, possibly linked to increased lipolysis. Reduced expression of *igf1* and *igf2* in muscle were seen in fish reared at 15°C, 17°C and 19°C compared with 13°C, showing reduced growth stimulation in the muscle already at 15°C, although with no differences in plasma IGF-1 (Hevrøy *et al.*, 2013). Kullgren *et al.* (2013) found no differences in plasma GH,

IGF or ghrelin in Atlantic salmon reared at different temperatures, however, an increase in plasma leptin was found in fish reared at 18°C, which could be connected to the reduced feed intake due to the anorexigenic effects (reducing hunger). Changes in plasma levels of metabolites also indicated a catabolic state in the fish reared at 18°C, as shown by several metabolites involved in energy metabolism, including alterations in lipid metabolism and reduced plasma levels of glutamine, tyrosine and phenylalanine (Kullgren *et al.*, 2013).

In Atlantic salmon and rainbow trout reared at 13°C or 19°C for 35 days, the concentrations of circulating IGF-1 were similar at both temperatures, however, rainbow trout had an increased concentration of IGFBP-1b, indicating a catabolic state at high temperature. Rainbow trout reared at 19°C also had lower expression levels of *igf1* and *igf2* in liver and *igf1* in white muscle, indicating reduced growth stimulation at high temperature (Hevrøy *et al.*, 2015). Atlantic salmon from the same experiment were studied 4–24 h postprandially, and the concentrations of plasma and muscle free amino acids were found to be lower in fish reared at 19°C compared with those reared at 13°C. Further, a lower expression level of *igf1* in muscle was observed, indicating lower growth stimulation, however, no temperature effect was seen on plasma IGF-1 protein level (Vikeså *et al.*, 2015). Plasma ghrelin has been shown to increase prior to an expected meal (preprandially), however, in this study no differences were detected in Atlantic salmon post-smolts reared at 19°C and 13°C (Vikeså *et al.*, 2015).

6.3.2 Temperature and hypoxia

Pörtner and Knust (2007) suggested that the fish tolerance to thermal extremes is limited primarily by a mismatch between the demand for oxygen and the oxygen supply to tissues. Increased water temperature leads to lower oxygen levels (Manasrah *et al.*, 2006), while at the same time the metabolic oxygen demand increases in fish. This can lead to a lack of oxygen or hypoxia, during periods of high water temperatures. Ventilation in fish is driven by the partial pressure of oxygen rather than of carbon dioxide as in terrestrial animals (Gilmour, 1997). Hypoxia therefore leads to hyperventilation, stress, reduced appetite and growth, and in the worst case death (Jobling, 1994; van Raaij *et al.*, 1996; Wang *et al.*, 2009). Primary stress responses

such as increased plasma levels of catecholamines, cortisol and lactate, and transcription of genes involved in ion regulation and respiration (Na^+/K^+ ATPase and hypoxia induced factor 1, HIF1) have been found in fish exposed to hypoxia (Borch *et al.*, 1993; Ellis *et al.*, 2002; Olsvik *et al.*, 2007). The stress response increases the energy consumption and, together with reduced appetite, hypoxia leads to reduced growth. This was found for small salmonids in fresh water (Brett and Blackburn, 1981; Dabrowski *et al.*, 2004) and for larger marine fish (Thetmeyer *et al.*, 1999; Pichavant *et al.*, 2000). In rainbow trout exposed to low oxygen levels (5.7 mg/l versus 9.3 mg/l), the maximal feed intake was reduced, while no differences were detected in the utilization of the ingested protein or energy (Glencross, 2009). Similar observations in growth depression while the feed conversion efficiencies were not affected were seen in juvenile halibut (40 g) and cod (*Gadus morhua*) exposed to a range of oxygen concentrations in long-term studies (Thorarensen *et al.*, 2010, 2017). These studies indicate that the feed intake is downregulated by low oxygen saturation. Such downregulation, with no effect on feed utilization, was confirmed in a feeding experiment that included Atlantic salmon reared under elevated temperature (17°C) and hypoxia (70% oxygen saturation) (Vikeså *et al.*, 2017).

Since moderate hypoxia leads to growth depression, it is recommended to oxygenate waters in tanks and sea to 100% to support growth and metabolism. Hyperoxygenation (> 150% oxygenation) at ambient normal temperatures does not necessarily promote growth, and it may rather contribute to elevated oxidative and metabolic stress, with reduced resistance to infectious diseases (Lygren *et al.*, 2000; Fridell *et al.*, 2007; Landis *et al.*, 2013). Since the water partial pressure of oxygen is lower at elevated temperatures, the overall risks and benefits of using hyperoxygenated water is unclear.

6.3.3 Triploid fish as a climate research model

There are economical, nutritional and ecological incentives for producing triploid fish for aquaculture (Piferrer *et al.*, 2009). Phenotypic and physiological differences between triploids and diploids have been discussed, and these include growth, smoltification, susceptibility to stress and infectious diseases, and production disorders (Fraser *et al.*,

2012). Generally, a triploid fish seems to be less robust to environmental changes than its diploid siblings. In rainbow trout reared in seawater, the negative impact of chronic high water temperatures on growth and survival of triploids was emphasized by Ojolic *et al.* (1995). Similarly, triploid Atlantic salmon showed lower tolerance to high temperature in both fresh water and seawaters, and elevated water temperature has been identified as one of the main challenges related to triploidy welfare (Hansen *et al.*, 2012; Sambraus *et al.*, 2017). At close to optimal temperatures (15°C), similar growth was seen in triploid and diploid Atlantic salmon, while at high water temperatures (19°C) lowest growth and highest mortality were seen in triploids (Migaud, 2011). Triploid Atlantic salmon reared at low (70%) oxygen saturation at elevated temperatures (19°C) had reduced appetite and growth, and elevated mortality compared with fish reared at 100% oxygen saturation (Fjelldal *et al.*, 2012a). Most studies show similar physiology between triploid and diploid fish from the same parents; however, triploid fish seem less robust towards suboptimal rearing and environmental conditions, such as elevated temperatures and hypoxia. Experiments with triploid fish may therefore serve as models to unveil the mechanisms behind temperature and hypoxia tolerance.

6.3.4 Starvation at elevated temperatures

The physiological response of fish under conditions of high temperature and hypoxia may in part be similar to that during periods of starvation, which in turn may be a coping strategy due to metabolic limitations at low oxygen supply. During such periods, the IGF system is depressed, and the fish utilize their body reserves to maintain basal metabolism and cope with external challenges. This is a catabolic state and consequently the fish lower their energy reserves and muscle growth. After re-administration of feed, the fish gradually recover their body reserves through increasing the feed intake, resulting in compensatory growth and an increase in the anabolic efficiency. One hypothetical way to alleviate the negative metabolic impact of thermal stress would be to starve fish under extreme sea temperatures. Atlantic salmon have a very high level of GHR in fast muscle tissue (Hevrøy *et al.*, 2007), are well adapted to longer periods of starvation during migrations, and may easily cope with food deprivation periods. In a long-term study on Atlantic

salmon, the fish (600 g) were reared at high temperature, with or without access to feed, and followed up during a recovery period. In total, there were minor beneficial effects of starving fish at 19°C compared with *ad libitum* feeding the fish at 19°C. The starved fish had better feed intake and nutrient retention during recovery at an optimal temperature (Normann, 2014).

6.4 Stress Responses at Different Biological Levels

6.4.1 Acute and chronic stress

The environmental changes involved in climate change (temperature, water gases, eutrophication, etc.) fluctuate naturally, and the fish can normally meet and adapt to these seasonal and diurnal variations adequately if these are within their tolerable limits. More extended and more rapid deviations may be stressful for the fish, and especially for farmed fish in a local and limited environment that cannot escape the stressors.

Water temperature and dissolved oxygen are the climate-related environmental factors that mostly affect growth and metabolic rate of fish (Brett, 1979), and are also interacting factors that set critical limits for the fish with respect to feed efficiency, growth, feed intake, metabolism and survival (Jobling, 1997). Rapid and chronic environmental exposures elicit acute and chronic stress responses and compensatory physiological measures, at a given energy cost for the animal. Depending on the adversity, the fish may enter a negative energy balance by higher metabolic costs, reduced feed intakes, impaired growth, immunosuppression and increased susceptibility to infectious diseases. Adult fish may be more sensitive to such thermal and hypoxic stresses than juveniles, as evaluated by growth reduction and survival (Vikeså, 2017).

Fish have the same neuroendocrine stress responses and secondary and tertiary physiological responses as terrestrial animals (Wendelaar-Bonga, 1997; Iwama, 1998). The secondary compensatory processes include behavioural response, mobilization of energy, and changes in blood chemistry and physiology, all to re-establish homeostasis. Plasma cortisol concentration has been used as the main indicator of stress, both in stress experiments and in practical farming (Ellis *et al.*, 2012). However, the usefulness of cortisol in a welfare perspective is questioned without considering it in a broader

perspective, including the interaction of psychological (behavioural, abnormal swimming, responsiveness, etc.) and given physiological distress components. Climate change normally includes interactions of several environmental conditions, with a complex set of primary and secondary resistance responses, which may end in individual exhaustion. In overall terms, elevated temperature reduces water oxygen concentration and increases carbon dioxide concentrations and metabolic wastes in fish farming (Wedemeyer, 1996). For example, below a critical level of dissolved oxygen, oxygen consumption will be restricted and at severely low concentration, lethal asphyxia and mortalities occur. The lethal oxygen level seems to be higher for cold-water species such as salmonids than warm-water species, however, depending on temperature (Wedemeyer, 1996). Feeding also reduces the oxygen levels during and after a meal (Wagner *et al.*, 1995). Low oxygen levels, as seen from elevated fish-rearing densities can act as a chronic stressor to fish; however, it is not clear which threshold values elicit the stress response (Ellis *et al.*, 2002).

Extreme temperatures ultimately lead from transition to anaerobic metabolism, with increased energy demands beyond the aerobic scope and increased oxidative stress. This leaves the cellular antioxidant and heat shock protein (HSP) defences important for molecular functions and ultimately fish survival (Pörtner, 2002). The stress response at the cellular level shows elevated levels of HSPs which have been proposed as an indicator of stressed states in fish (Iwama *et al.*, 2004). Various HSPs are upregulated in response to a wide variety of stressors, as they have a role in protection, as well as repair and degradation of misfolded or denatured proteins (Welch, 1993; Freeman *et al.*, 1999; Rabergh *et al.*, 2000).

Increased water temperature and anaerobic metabolism means increased catabolism of amino acids. Besides increased metabolism with higher temperature, Jürss (1979) showed temperature-dependent transaminases in fish, responsible for deamination of amino acids to keto acids. At the metabolic level, juvenile rainbow trout that were exposed to elevated temperature (20°C) for 10 weeks had a lower concentration of energy-rich phosphocreatine and ATP, a higher concentration of AMP (adenosine monophosphate), while no effects were seen on ADP (adenosine diphosphate) (Viant *et al.*, 2003). Changes in plasma metabolites in fish exposed to 18°C, compared with 12°C and 8°C, supported a

catabolic state (Kullgren *et al.*, 2013). In a comparative study, where similar sized Atlantic salmon and rainbow trout were exposed to water temperatures of 19°C compared with optimal levels of 13°C, Hevrøy *et al.* (2015) found increased plasma glucose concentration despite a similar feed intake. Clearly, an elicited stress response is part of the physiological and psychological coping of the farmed fish experiencing extreme climate change, such as elevated temperatures and hypoxia.

6.4.2 Oxidative stress

All life-bearing biochemical processes include redox reactions. These reactions, if out of control may result in oxidative stress to the organism or cells and can cause oxidative damage to cellular components (DNA, lipids and proteins). Oxidative stresses have been identified as basic reasons for a multitude of human lifestyle disorders such as cancer, cardiovascular disease, cataracts and Alzheimer's disease (Spector, 2000). Similarly, in aquaculture, lipid oxidation and lipid liver degeneration are classical examples of oxidative disease in salmonids (Waagbø, 2006). The disorder was prevented by supplementation of antioxidants, such as vitamins C and E, and selenium (Waagbø *et al.*, 2001; Waagbø, 2006).

Elevated temperature imposes oxidative stress, both from the direct impact of temperature on the cell structures and biological systems, and from increase in reactive oxygen species (ROS) production from the accelerated energy metabolism (Fig. 6.1). These reactive compounds are produced in small amounts in the normal cell metabolism, and have important roles in cell signalling and in immune responses (Cadenas, 1989). The intracellular concentration of ROS is influenced by production and clearance rates, and the clearance is influenced by antioxidants. Antioxidants delay or inhibit oxidation of cell constituents by being a more easily oxidized substrate or through reducing oxidants and ROS into products that are more easily metabolized by the cell (Fig. 6.1). Antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been detected in most fish species (Ken *et al.*, 2003; Martínez-Álvarez *et al.*, 2005).

Glutathione (GSH) is the main non-protein thiol in the cells, and acts as an endogenous antioxidant by scavenging free radicals in addition to being a cofactor for GPx (reviewed by Lu, 2013). During

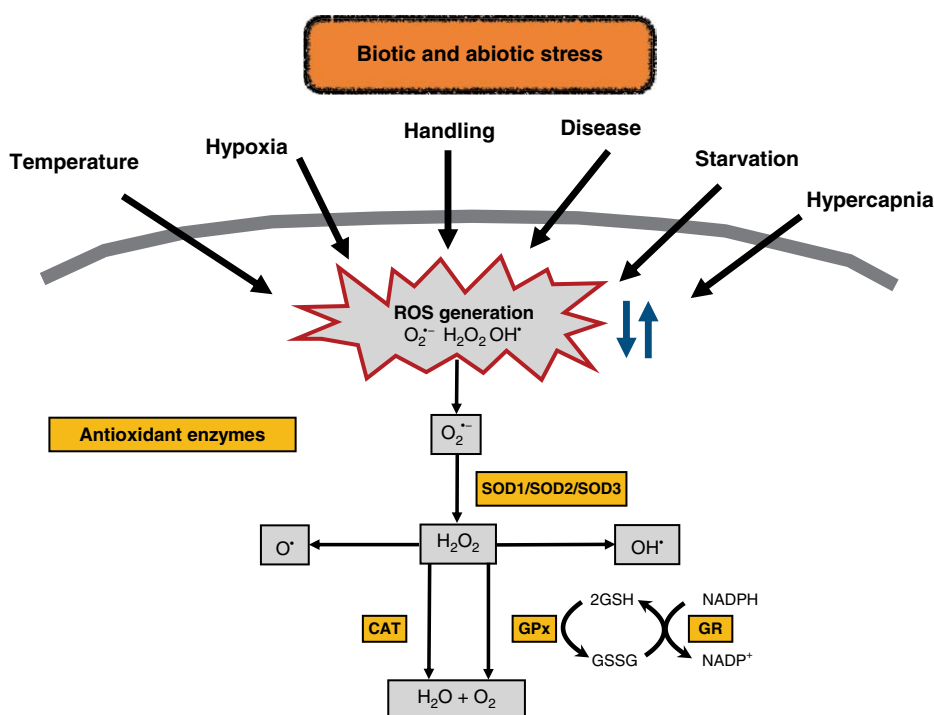


Fig. 6.1. Biotic and abiotic stress may change the cellular levels of reactive oxygen species (ROS). CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase.

changes in temperature, the cellular metabolism is reorganized in fish, and the activity of GSH-dependent enzymes have been shown to increase in goldfish tissues, along with higher levels of thiols such as GSH (Lushchak and Bagnyukova, 2006; Bagnyukova *et al.*, 2007). However, a reduced pool of GSH will compromise the peroxide or free radical defence system that can lead to non-enzymatic oxidation of cellular lipids, protein and nucleic acids, and result in cell death.

If the balance between ROS production and elimination is disturbed, the elevated concentration of ROS results in an upregulation of genes that code for antioxidant enzymes and it maintains the redox homeostasis (Dröge, 2002; Fig. 6.1). In a study with Atlantic salmon (1.6 kg) maintained at 13°C, 15°C, 17°C and 19°C for 45 days, there was reduced expression of several hepatic genes encoding antioxidant proteins at temperatures of 17°C or above (Olsvik *et al.*, 2013a). Reduced expression of *cuznsod*, *mnsod*, *gpx1* and *gr* (glutathione reductase gene) at suboptimal temperature (only a few degrees above the optimal temperature for growth) suggests that oxidative stress markers are sensitive

indicators of an imbalance in ROS generation in salmon liver cells. At 19°C, temperature stress downregulated several key transcription factors such as MYCN, HIF1A, hepatocyte nuclear factor (HNF)1A, HNF4A and nuclear factor erythroid derived 2-like 2 (NFE2L2), suggesting that heat stress reduces the transcriptional rate in the salmon liver. The results indicate a switch towards increased transcription of protective enzymes at the cost of synthesis of maintenance enzymes. The study demonstrates how transcription of antioxidant genes provide useful markers of temperature-induced stress in fish, in line with effects caused by a number of environmental stressors in fish (Lushchak, 2011, 2016).

Among the lipid-soluble antioxidants, tissue vitamin E status has been shown to decline under elevated thermal conditions in Atlantic salmon (R. Waagbø, 2010, unpublished observations). The reason for the lowered vitamin E status is not clear, however, lower vitamin E retention at high temperature may represent a net use of vitamin E as an antioxidant. In carp (*Cyprinus carpio*), the muscle vitamin C status reduced at high temperature, along

with higher concentration of thiobarbituric acid reactive species (TBARS) and lower concentration of GSH, however, these levels were normalized in fish given surplus dietary vitamin C (Hwang and Lin, 2002). Similarly, liver lipid peroxidation in thornfish (*Terapon jarbua*) exposed to high temperature was reduced by vitamin C-supplemented diets. Dietary supplementation of vitamin C has been shown to prevent vitamin E deficiency through regeneration of vitamin E (Hamre *et al.*, 1997), thus increasing the dietary vitamin C concentration may improve or sustain the vitamin E status at oxidative stress. Altogether, there seems to be potential to improve the resistance to thermal stress, by increasing the dietary supply of antioxidant vitamins.

Histidine (His) and its derived dipeptides anserine (Ans) and carnosine (Car) are multifunctional compounds, among other important water-soluble antioxidants (Kohen *et al.*, 1988; Wade and Tucker, 1998; Velez *et al.*, 2008). Increasing the dietary histidine concentration increases the concentration of His, Ans and Car in salmonid tissues, and also influences the antioxidant defence system in Atlantic salmon liver (Remø *et al.*, 2014) and in lenses supplemented with His *ex vivo* (Remø *et al.*, 2011). Muscle Car acts as an immunity modifier and antioxidant, both directly and indirectly through influencing the endogenous antioxidant system (Boldyrev, 2012). Elevated temperature seemed to increase the His requirement needed to prevent cataract development in both diploid and triploid Atlantic salmon (Sambras *et al.*, 2017). However, the exact role of the histidine compounds in the integrated antioxidant system in different farmed fish species is not clear and needs further study.

6.4.3 Heat shock proteins (HSPs)

HSPs are stress proteins and are considered an important part of the cellular stress response (Santoro, 2000). HSPs are a highly conserved family of related proteins whose expression is increased when cells are exposed to elevated temperatures (Basu *et al.*, 2002). The upregulation of HSPs is known as the heat shock response and is induced primarily by thermal stress (Wu, 1995). HSP27, HSP70 and HSP90 (named according to their molecular weight) have been extensively studied (Feder and Hofmann, 1999; Feidantsis *et al.*, 2009). In some species (e.g. in Chinook salmon and rainbow trout cell lines), HSPs are upregulated after

thermal stress and a prominent response is the increase in HSP70 levels (Iwama *et al.*, 1998, 1999). However, in other species such as Atlantic cod or Antarctic notothenioid fish (*Trematomus bernacchii*), there was no heat shock response in thermal stress (Hofmann, 2005; Zakhartsev *et al.*, 2005). Furthermore, different species and strains (e.g. goby and stream fishes) of the same species may have different patterns in their tolerance to heat stress (Iwama *et al.*, 2004).

Many studies on heat shock response to thermal stress have been conducted in Salmonidae (Lund *et al.*, 2002; Feldhaus *et al.*, 2010). HSP70 was significantly induced in hepatic and branchial tissue of juvenile salmon subjected to a 15 min heat shock at 26°C (DuBeau *et al.*, 1998). Zarate and Bradley (2003) also reported that HSP70 and *hsp90* mRNA levels in Atlantic salmon increased threefold and twofold above control levels (at 15°C) following 15-min heat stress at 26°C. In rainbow trout, Fowler *et al.* (2009) found that HSP70 was significantly higher in the hearts of fingerlings than in adults following an acute (1 h) heat stress at 25°C. However, most of these studies focused on the acute heat stress and less is known about the effect of chronic heat stress exposures experienced by farmed salmonids. Lindquist (1986) pointed out that different proteins might be involved in protecting cells from short-term exposures and chronic exposures to high temperatures. A yellowtail (*Seriola quinqueradiata*) tailfin cell line was used to demonstrate temperature regulation in fish, by use of stress responsive markers of the HSP70 family (Yabu *et al.*, 2011). These workers concluded that chaperone-mediated autophagy was assisted by both heat shock cognate protein (HSC)70 and HSP70 in the lysosomes, which may relate to adaptation and survival of the exposed cells. In adult Atlantic salmon (1.6 kg) maintained at 13°C, 15°C, 17°C and 19°C for 45 days, *hsp90b* was the only HSP gene that was upregulated by heat stress, while no effects were seen on *hsp70* transcription (Olsvik *et al.*, 2013a).

Post-smolt Atlantic salmon (100 g) at 19°C for 35 days showed a transient heat shock response in white muscle HSP70 compared with fish reared at 13°C, but not in liver tissue, while rainbow trout were less affected (Dr Han Dong, 2012, unpublished observations). However, when Atlantic salmon were transferred from 13°C to 19°C, gene transcription of HSP70, HSP90, HSP27, HIF1A and glucose regulated protein 94 (GRP94) and protein expression of HSP70 were induced in liver

tissue. Takle *et al.* (2005) confirmed that liver, kidney and gill seemed to be more sensitive to heat shock than other tissues. These results suggest that Atlantic salmon is more sensitive to heat stress than rainbow trout when acutely exposed to water temperatures up to 20°C, simulating realistic changes in salmonids farming conditions.

6.4.4 Cell membrane temperature adaptations

At the cellular level, temperature alters the velocity of chemical and enzymatic reactions, rates of diffusion, membrane fluidity and macromolecule stability in fish (Guderley, 2004), and has a direct impact on feed intake, metabolism, protein utilization and therefore on the growth efficiency of fish (Jobling, 1997). The cell membranes acclimatize to changes in water temperature by homeoviscous adaption (Waagbø, 2006), and this implies shifting lipid class structures and fatty acid composition of the phospholipids in the cell membranes to fit the ambient temperature. Generally, membranes increase the fractions of unsaturated fatty acids and monoene fatty acids and decrease saturated fatty acids during cold acclimatization (Hazel and Williams, 1990). Similarly, Miller *et al.* (2006) showed that there were adaptations in the cell membrane structure in Atlantic salmon at high temperatures (19°C versus 15°C), with elevated levels of saturated fatty acids in the membranes (polar fractions) of gills and white muscle, coinciding with lower n-3 and n-6 polyunsaturated fatty acids. Tissue fatty acid composition is also affected by dietary lipids, with implications for fish physiology and immunity (Waagbø, 2006). Thus, at the cellular level, farmed fish adapt cell membranes both according to the feed lipid sources and the ambient water temperature. Any major conflicts between dietary fatty acid composition from novel feed ingredients and the optimal membrane composition may therefore result in welfare issues, such as reduced immunity and resistance to bacterial diseases (Sheldon and Blazer, 1991; Waagbø *et al.*, 1993a, b; Waagbø, 2006).

6.4.5 Tissue buffering capacity

Histidine and its derivatives are present in free forms in very high concentrations in salmonid tissues. At the physiological pH range of the cold water fish muscle (pH 6.5–7.5), the buffering capacities of the His dipeptides are very high and

higher than that of free His (Abe and Okuma, 1991). Similar to pelagic fish species such as scombroids, Atlantic salmon seem to rely on His and His-related compounds to buffer protons produced by high anaerobic activity in white muscle tissue (Suzuki *et al.*, 1987, 1990). Compared with His, the buffering capacity of Ans seems to be constant within the rearing temperature range in normal salmonid farming (Abe and Okuma, 1991), and the buffering capacity can be increased by increasing the dietary His levels (Ogata, 2002).

At critical high temperatures, the aerobic scope is lower, resulting in a switch to anaerobic energy production (Pörtner and Farrell, 2008) and consequently a higher usage and requirement for proton buffers such as His and Ans (Abe, 2000). The principle of how chemical groups like the imidazole histidine can maintain their buffering function independent of temperature-related pH changes is called the ‘alphastat hypothesis’, and explains how cellular protein structure and functions can be conserved within a wide range of pH in fish and ectothermic vertebrates (Burton, 2002). For example, Van Dijk *et al.* (1999) examined the temperature-mediated changes in metabolism in Antarctic eelpout (*Pachycara brachycephalum*) with increasing temperature and demonstrated higher increase in standard metabolic rate than in eelpout from the more temperate North Sea (*Zoarces viviparus*). The white muscle intracellular pH change followed the alphastat regulation in the range of 3–24°C, with a moderate decline of only -0.016 pH units per degree Celsius (°C) (total 0.34 units). Above this temperature range, pH deviated from this development, indicative of an acid–base disturbance. From this comparative study and available literature data they suggested that the final cause of heat death was impaired respiration in combination with circulatory failure (van Dijk *et al.*, 1999).

The metabolic importance of the high concentrations of the free imidazole concentrations for climate-related anaerobic metabolism have not been clearly elucidated. Remø *et al.* (2012, unpublished) demonstrated a reduction in muscle Ans levels in Atlantic salmon parr and smolt related to severe environmental hypercapnia. However, Hevrøy *et al.* (2013) observed that muscle Ans increased linearly with increasing temperature from 13°C to 19°C. In an earlier study Hevrøy *et al.* (2011) found that all indispensable amino acids were reduced after 48 h and 14 days starvation except for histidine, which was not altered. In addition,

Ans was maintained at a stable level in muscle, which supports a priority for keeping a high buffer and stable cell osmotic status (Breck, 2004). Geda *et al.* (2017) indicated that the different muscular storage of free amino acids and imidazole compounds found in fish species such as Nile tilapia (*Oreochromis niloticus*) and carp determines the metabolic reaction of fish species to elevated temperatures, like differential lipid and amino acid mobilization for energy purposes.

6.4.6 *In vitro* models to study climate change stress

The number of vertebrate animals used in research increases from year to year (Taylor *et al.*, 2008). The principles of the 3Rs (Replacement, Reduction and Refinement), developed more than 50 years ago, aim to reduce the use of experimental animals (Russell and Burch, 1959). As a result, different methods and alternative organisms are applied to address important scientific questions without the use of animals. *In vitro* models are increasingly being used in biological examinations, and can successfully be applied in climate change research. Besides decreasing animal numbers, *in vitro* models offer reduced costs of fish maintenance and care, reduced use of equipment, shorter exposure time, and increased throughput for evaluating multiple experimental conditions. In particular, *in vitro* systems allow the study of mechanistic insights into disease, and provide efficient platforms for screening of the mode of action of chemicals and the examination of dose–response relationships (see Iwama *et al.*, 1999).

Farmed Atlantic salmon has been associated with legacy contaminants such as dioxins, PCBs (polychlorinated biphenyls) and DDT (dichlorodiphenyltrichloroethane) (Hites *et al.*, 2004), mainly originating from the marine feed ingredients such as fish oil. Screening of newer feeds, in which the content of marine ingredients has been replaced with increasing levels of plant-based material, has revealed occurrence of agricultural pesticides (Nácher-Mestre *et al.*, 2014). Considerable attention has been paid to interactions between climate change and contaminants in recent years (Noyes *et al.*, 2009). In ectothermic organisms such as fish, it is often suggested that toxicity increases with increasing temperature. The upper temperature tolerance limits can thus be lower in the presence of certain contaminants (Cossins and Bowler, 1987; Patra *et al.*, 2007).

In an attempt to address chemical vulnerability in fish acclimatized to suboptimal high temperature, Olsvik *et al.* (2015, 2016a, b) examined the toxicity of a mining chemical (dadmac, the building block of polydadmac) and two heavy metals (Cd and Hg) in cells obtained from heat-acclimatized Atlantic salmon post-smolt. Groups of salmon were adapted either to optimal growth temperature (15°C) or to high suboptimal temperature (20°C) for 3 months before cells were harvested for the *in vitro* experiments. The first experiment showed that while fish acclimatization temperature had a significant effect on the basic transcriptional levels of several stress-responsive genes (*gpx1*, *mta*, *tf*, *hsp70*, *bclx*, *mapk1*, *atp7a* and *fads2*), no effect on fish pre-acclimatization temperature was seen on dadmac toxicity (0 µM, 1.0 µM, 100 µM) in terms of cytotoxicity and transcription of selected genes (Olsvik *et al.*, 2015). In the second and third experiment, one additional group of salmon was added, adapted to high temperature (20°C) and fed a diet spiked with surplus antioxidants (vitamins A, C and E, selenium, zinc and histidine), to examine if antioxidants could ameliorate the chemical toxicity in cells from temperature-stressed fish. The antioxidant-spiked diet changed the levels of vitamin C, vitamin A1, vitamin A2, Se, Fe and Cu in whole fish or liver of temperature-stressed fish, while fish pre-acclimatization temperature affected the concentration of Cd and As (Olsvik *et al.*, 2016a). In cells from salmon acclimatized to 15°C and 20°C and exposed to 0 µM, 1.0 µM, 100 µM Cd, metal-induced cytotoxicity was more pronounced in cells from fish pre-acclimatized to a high temperature than in cells from fish grown at optimal temperature. The diet spiked with antioxidants could not ameliorate the Cd-induced cytotoxicity in cells from temperature-stressed fish. At the transcriptional level, fish pre-acclimatization temperature had an effect on the basic levels of five genes (*cat*, *gsr*, *hsp70*, *tf* and *fth1*). Cadmium exposure affected 11 examined genes, of which most were linked to oxidative stress. The transcriptional levels of the majority of the altered genes were changed in cells harvested from fish grown at 20°C compared with 15°C. Interaction effects between Cd exposure and fish pre-acclimatization temperature were seen for four genes – *hmox1*, *mapk1*, *fth1* and *mmp13*. Overall, the study showed that cells from temperature-stressed fish were modestly more vulnerable to Cd stress, and this indicates

that mechanisms linked to oxidative stress may be differentially affected in temperature-stressed cells. The results from the Hg exposure study showed no impact of fish pre-acclimatization temperature on metal-induced cytotoxicity (Olsvik *et al.*, 2016b). Fish pre-acclimatization temperature had an effect on the basic levels of five genes (*cat*, *gpx1*, *mt-a*, *hsp70* and *tf*). However, even if spiking the diet with antioxidants resulted in higher concentrations of Se and vitamin C and reduced the concentration of Hg in the liver *in vivo*, no interactions were seen between fish pre-acclimatization temperature or the dietary supplementation of antioxidants and Hg toxicity *in vitro*. No evidence was therefore found to suggest that inorganic Hg is more toxic in cells harvested from temperature-stressed fish. These studies demonstrate how *in vitro* models can be used to assess the impact of climate change on chemical toxicity in fish.

6.5 Nutritional Disorders Related to Climate Change

Production-related disorders are recurrent in fish farming, and they are often related to major changes in feed composition and environmental conditions, handling, medication or their interactions (Waagbø, 2006, 2008). As has been discussed above, major challenging environmental parameters arising from climate change dramatically affect fish physiology and coping mechanisms, and so it is no wonder that fatal interactions may occur in fish farming which is subject to so many variables. Eye, bone, gill and skin tissues are among the sensitive organs in which welfare issues can be easily visualized as arising from climate changes.

6.5.1 Cataracts

Cataracts represent an important welfare and ethical problem in the intensive farming of Atlantic salmon. During cataractogenesis, both the lens epithelium and the fibres may become damaged, and the resulting opacities reduce vision causing potential blindness. The aetiology of cataracts in farmed fish is multifactorial and can result from suboptimal diets, genetic factors and environmental challenges (Bjerkås *et al.*, 2006), including periods with fluctuating temperatures and temperature extremes (Fig. 6.2). Increasing temperatures within temperature optimum stimulates growth and several studies have shown a correlation between rapid growth rates and cataract development (Bjerkås *et al.*, 1996; Waagbø *et al.*, 1996; Breck and Sveier, 2001).

Nutritionally related cataracts have been observed in several fish species, and have been associated with suboptimal dietary levels of methionine, tryptophan, riboflavin, zinc and manganese, often in relation to new raw materials in the feed (Bjerkås *et al.*, 2006). Waagbø *et al.* (2003) also showed that a balanced diet of pro-antioxidants and antioxidants is important to prevent cataract development in Atlantic salmon. However, the focus in recent studies has been on the essential amino acid histidine. It was shown that the requirement to minimize cataract development in Atlantic salmon after seawater transfer was nearly twice the previously defined requirement for growth (NRC, 2011), and estimated to be 13.4g His/kg feed (Remø *et al.*, 2014). Histidine is taken up by the lens and used to synthesize N-acetyl-histidine (NAH) and the severity of cataracts is negatively correlated with the concentration of NAH in the

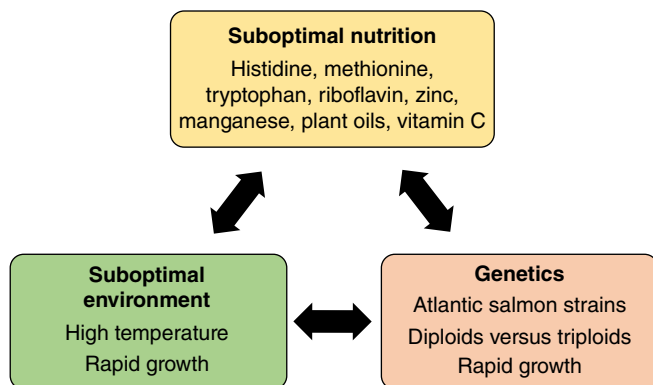


Fig. 6.2. Risk factors for cataract development in Atlantic salmon. The risk imposed by a suboptimal environment covers climate changes.

lens (Waagbø *et al.*, 2010; Remø *et al.*, 2014). NAH has been shown to have important roles as an osmolyte (Rhodes *et al.*, 2010), buffer component (Breck *et al.*, 2005) and possibly intracellular antioxidant (Remø *et al.*, 2011) in the lens and a certain concentration is therefore essential in maintaining the lens water balance and cell integrity.

Waagbø *et al.* (2010) showed a natural increase in seawater temperatures (12–18.5°C) resulted in a higher prevalence of cataracts in adult Atlantic salmon. In this study, a dietary histidine concentration of 12.8 g/kg feed reduced the severity of cataracts, similarly to 17.9 g/kg feed, indicating that the requirement to minimize cataract development after seawater transfer may also be sufficient in periods with high temperature. In experiments on Atlantic salmon raised at lower temperatures (8–9°C), low cataract scores were found, despite lower dietary His concentrations (Trøpe *et al.*, 2010; Remø *et al.*, 2011). Thus, it appears that the requirement for His to reduce the risk of cataract development in Atlantic salmon is dependent on the rearing temperature, and possibly due to lower growth rates at lower temperature. The increased requirement for His at high temperature is further supported by a comparative study between diploid and triploid Atlantic salmon reared at 10°C versus 16°C. In this study, cataract development was significantly higher in both ploidies at 16°C compared with 10°C in fresh water, irrespective of dietary His concentration (Sambraus *et al.*, 2017). However, at high temperature a dietary concentration of 13g His/kg feed significantly reduced the severity of cataracts compared with 10 g His/kg feed, while also showing that triploid salmon are more susceptible to cataract development at high temperature compared with diploid salmon.

The mechanisms for the increased risk of cataract development have not been determined (Waagbø *et al.*, 2010; Sambraus *et al.*, 2017), however, increased oxidative pressure and an alteration in nutrient requirement at high temperature are hypothesized to impact cataract development. In a comparative study between rainbow trout and Atlantic salmon post-smolts reared at 13°C or 19°C for 35 days, no differences were seen in the severity of cataract development due to temperature, however, temperature influenced the global metabolic profile in the lenses, which may explain the higher risk of cataracts at high temperature (Remø *et al.*, 2017). The metabolic profile indicated that high temperature alters the osmoregulatory ability, carbohydrate metabolism and redox

regulation in the lenses. In this study, both species were given a common feed with 10 g His/kg feed, which was insufficient to maintain eye health for Atlantic salmon at both temperatures, while it appeared to be sufficient for rainbow trout which was able to maintain a cataract-preventive concentration of NAH in the lens. The concentration of NAH was, however, significantly lower in lenses from rainbow trout reared at 19°C compared with 13°C.

Osmotic and often reversible cataracts are more commonly seen due to osmotic disturbances such as after seawater transfer of Atlantic salmon (Bjerkås *et al.*, 2006). A reduced level of lens osmolytes at high temperature indicates that high temperature may influence the osmoregulatory ability of lenses which may contribute to the formation of irreversible cataracts. In Atlantic salmon lenses, alterations in intermediates in the glucose breakdown pathways suggest a temperature-dependent dysfunction or an overload of glycolysis at high temperature (Remø *et al.*, 2017). A higher level of sorbitol was found in salmon lenses reared at 19°C, which could result in osmotic stress and consequently contribute to cataract formation, as in hyperglycaemic animals and humans (Varma *et al.*, 1979; Chan *et al.*, 2008). Atlantic salmon reared at 19°C had a higher plasma glucose concentration (Hevrøy *et al.*, 2015), which was previously associated with osmotic cataracts in salmon (Waagbø *et al.*, 2003). Thus, heat-induced hyperglycaemia may influence the lens' water balance by influencing the lens' carbohydrate metabolism.

Oxidative stress plays an important role in cataract development in animals including humans (Spector, 1995; Williams, 2006), and the lens is especially vulnerable to decreased protein turnover towards the lens native nucleus (Brennan and Kantorow, 2009). Crystallins (lens proteins) contain high levels of thiol groups; therefore it is important to have a balanced redox state to maintain transparency (Lou, 2003). In young lenses, the thiols are protected by being sheltered in the macromolecular structures, but as the thiols over time become exposed, proteins, GSH and methionine may be oxidized. This leads to the formation of high molecular-weight protein aggregates, linked by disulfide bridges, that cause light scattering and loss of transparency (Spector, 1995). In rainbow trout lenses, a higher level of both reduced GSH and oxidized glutathione disulfide (GSSG) at 19°C compared with rainbow trout reared at 13°C indicated that rainbow trout were able to increase the defence against oxidative stress in the lens and thereby

possibly maintain the redox homeostasis in the lenses. In comparison, the Atlantic salmon lenses had large variations in the GSH level indicating individual differences in the GSH metabolism and the ability to regenerate GSH, resulting in a lower ability to cope with oxidative stress (Remø *et al.*, 2017). In this study, both species had a higher level of ophthalmate in the lenses at 19°C compared with 13°C, and ophthalmate has been suggested to be a marker for GSH depletion and oxidative stress (Dello *et al.*, 2013). Thus, the risk of cataract development at high temperature may be related to increased metabolic and oxidative stress.

6.5.2 Skin health

Temperature, oxygen and salinity may impact the skin health under commercial farming, in addition to physical handling and treatments (Jensen, 2016). Both low and elevated temperatures affect skin structures and related cells, and skin infections and ulcers easily appear at both low (Lillehaug *et al.*, 2003; Karlsen *et al.*, 2012; Bornø and Lie Linaker, 2015) and elevated temperatures (Bruno *et al.*, 2013), and often in combination with low oxygen levels, handling and stress. Secondary welfare conditions to skin disorders and scale losses often appear as: (i) osmotic disturbances; (ii) increased susceptibility to secondary infections (Bruno *et al.*, 2013), including ectoparasites such as the salmon louse; and (iii) mortalities.

The appearance of ulcers has been observed in extreme water temperatures or where there have been temperature fluctuations, and is among the so-called production related diseases (Waagbø, 2008). The skin physiology and immunology are sensitive to both low and high temperatures (Jensen *et al.*,

2015a), and are related to lower resistance to infectious diseases like the bacteria causing winter ulcer and infections with salmon lice. Such challenges may be more frequent in future intensive fish farming due to climate change.

Optimization of selected nutrients in the diet counteract the development of ulcers and skin disorders and improve wound healing by modulating both immune functions and the molecular processes of collagen formation (Waagbø, 2008). Among the micro-nutrients, surplus feed supplies of vitamins C and E, and zinc, are the most studied in relation to skin disorders and repair in farmed fish (Wahli *et al.*, 2003; Waagbø, 2008; Fountoulaki *et al.*, 2010; Jensen *et al.*, 2015b). Temperature-related skin changes in healthy Atlantic salmon smolt included changes in skin composition, histology and immune-related gene transcripts (Jensen *et al.*, 2015a). From rearing temperatures of 4°C, 10°C and 16°C, the antioxidant vitamins C and E increased in the skin, epidermal thickness decreased, mucous cell areas increased, and a number of HSP genes were upregulated. Wound healing also increased with temperatures between 4°C or 12°C in salmon post-smolt, while higher temperatures were not examined (Jensen *et al.*, 2015b). This illustrates that antioxidants may be mobilized to the skin, where they are needed at elevated temperatures.

In a study where Atlantic salmon smolt were exposed to temperatures (4°C, 10°C, 15°C and 20°C) for 60 days, fish kept at the highest temperature eventually developed skin ulcers (R. Waagbø, 2000, unpublished data). From the variation in status of single B vitamins in muscle (Table 6.1), metabolism was clearly affected. It is, however, difficult to say if supplementation of individual B vitamins in excess could be beneficial for the metabolism and skin health at elevated temperatures.

Table 6.1. Change in B vitamin concentration in liver and muscle of Atlantic salmon smolt fed a commercial feed for 60 days at 10°C and 20°C.

Vitamin	Liver status (10°C → 20°C)	Muscle status (10°C → 20°C)	Metabolism
Thiamine	Decreased 5%	Increased 27%	Towards anaerobic
Riboflavin	No change	Decreased 36%	Towards anaerobic
Niacin	Increased 50%	Increased 30%	Towards anaerobic
Biotin	Increased 150%	No change	Redistribution (?)
Pantothenic acid	Decreased 30%	Decreased 33%	Towards anaerobic
Pyridoxine	No change	No change	Trans-deamination
Folate	Increased 22%	Decreased 67%	Redistribution
Vitamin B12	No change	Decreased 31%	Towards anaerobic/redistribution

6.5.3 Bone deformities

Bone deformities have traditionally been linked to both suboptimal nutrition and nutritional-related toxicities, and results in visible malformations; however, high water temperatures have also been identified as a risk factor (Wargelius *et al.*, 2010; Ytteborg *et al.*, 2010; Grini *et al.*, 2011). Nutritionally related deformities have been associated with vitamins A, D, K and C (ascorbic acid), and also bone minerals such as calcium, phosphorus, magnesium, manganese, zinc and copper, either due to deficiencies or toxic levels (reviewed by Waagbø, 2008). Deformities (see Chapter 3, this volume) can be detected at the hatchery stage, usually in the form of neck deformities, vertebral and spinal disorders, due to alterations in the bone formation, resorption and mineralization processes (reviewed by Lall and Lewis-McCrea, 2007). Common deformities in farmed salmon are lordosis (saddleback), scoliosis (lateral and rotated curved vertebrae), kyphosis (hunchback), 'short-tails' (shortening of spinal column) and 'star-gazers' (bent neck) (Lall and Lewis-McCrea, 2007; Waagbø, 2008; Witten *et al.*, 2009; Fjelldal *et al.*, 2012b) and these affect swimming behaviour, feed uptake, energy utilization and disease resistance. Vitamin C deficiency is especially linked to the prevalence of lordosis and scoliosis, caused by insufficient hydroxylation of protein-bound proline and lysine and consequently reduced cross-linking of collagen in connective tissues (Sandnes, 1991; Waagbø, 2008). This may be of importance during periods with high temperature due to increased utilization/lower retention of vitamin C. Common carp reared at 35°C compared with 25°C had a lower hydroxyproline:proline ratio and collagen level, and these levels were increased by surplus dietary vitamin C at both temperatures (Hwang and Lin, 2002). Dietary phosphorus deficiency causes both reduced bone mineralization and skeletal abnormalities in salmon (Fjelldal *et al.*, 2012b, 2016), and has been shown to inhibit mineralization and enhance deformity in haddock (*Melanogrammus aeglefinus*) (Roy and Lall, 2007). Recent studies have shown a beneficial effect on bone health of increasing the dietary phosphorus and micronutrient concentration at high temperature in Atlantic salmon (Fjelldal *et al.*, 2016), especially in triploid salmon, which seems to be more sensitive to externally initiated production related disorders.

High water temperatures during different life stages of fish increase the prevalence of developmental defects and bone deformities. For example:

- jaw deformity in golden pompano (*Trachinotus ovatus*) larvae (Ma *et al.*, 2016);
- cranial deformities (Georgakopoulou *et al.*, 2007), deformed opercula bones, hyoid system, skull and jaw bones and spinal columns in sea bass (*Dicentrarchus labrax* L.) (Abdel *et al.*, 2004);
- body deformities (scoliosis, lordosis and bended operculum in juvenile tench (*Tinca tinca*) (Kaminski *et al.*, 2017);
- cranium malformations, abnormal body curvatures in gilthead sea bream (*Sparus aurata*) larvae (Pimentel *et al.*, 2016); and
- vertebral deformities (Grini *et al.*, 2010; Ytteborg *et al.*, 2010) and warped gill opercula, fin and jaw deformities in Atlantic salmon (Ørnsrud *et al.*, 2004).

High water temperatures may increase growth and development at early life stages (Ytteborg *et al.*, 2010), or fish may be reared in seawater with high temperatures, thereby increasing the risk of bone deformities (Grini *et al.*, 2011). Ørnsrud *et al.* (2004) found a higher prevalence of warped gill opercula, fin and jaw deformities, but not spinal deformities in adult Atlantic salmon (1.3 kg) exposed to 14°C compared with 8°C during the egg incubation stage. This shows the importance of temperature control during the early stages. Atlantic salmon raised at high temperature (16°C) had a higher growth rate, and higher prevalence of spinal column deformities than those raised at 10°C. The increased risk was linked to transcriptional changes in genes involved in maturation and mineralization of osteoblasts, as well as chondrocyte hypertrophy (Ytteborg *et al.*, 2010). The positive temperature effect on growth may also influence gene transcription in osteoblasts and chondrocytes, resulting in alterations in the vertebrae structure and composition and disruption of bone and cartilage production (Ytteborg *et al.*, 2010).

Grini *et al.* (2011) studied the effect of vaccination with a commercial multivalent oil adjuvant vaccine (Norwax from Intervet Norge AS) and bone deformation at two temperatures (10°C and 16°C in fresh water, and fresh water–seawater, and cross-over after seawater transfer 16–10°C, 10–16°C) in under yearling Atlantic salmon smolt (36 g). After the temperature regime experiment, the fish were

kept for 42 weeks until harvest size in a common net pen with ambient seasonal temperature variations between 8°C and 16°C. The early temperature regimes influenced growth, and the differences were still present at termination, showing a significant negative long-term effect of high temperature in the period after seawater transfer. The fish reared at 16°C during the first 6 weeks after seawater transfer also had a higher prevalence of externally detectable deformities with 20.3% compared with 3.2% in 10°C, and 91% of fish had one or more malformed vertebra compared with 36% and 62% in fish reared at 10–10°C or 10–16°C, respectively. The vertebral bone mineral content was similar in all experimental groups (40% Ca, 21% P), but decreased during the first 6 weeks in seawater by 10–15% in all groups, to levels regarded as deficient. Serum phosphorus levels also decreased during the first 3 weeks but were restored at 6 weeks. Thus, the low bone mineral content may have contributed to the increased risk of bone deformities at high temperature. Transcriptional analysis of the vertebrae showed that MMP-13 (matrix metalloproteinase 13), an indicator of bone degradation, was upregulated 3 weeks and 6 weeks after seawater transfer, showing a possible link between insufficient mineralization and increased bone degradation at high temperature (Wargelius *et al.*, 2010).

6.6 Nutrient Requirements in a Changing Climate

The future expansion in Atlantic salmon production and aquaculture in general calls for increased production of fish feeds. Due to the limited availability of fish meal and fish oil, the use of non-marine raw materials in commercial diets have doubled since the 1990s, where 70% of grower feeds (both proteins and lipids) are of plant origin (Ytrestøyl *et al.*, 2015). The replacement of fish meal with plant proteins is possible if the content of essential amino acids and all other essential nutrients are met. The former can be done by mixing protein sources and supplementation of crystalline amino acids (Espe *et al.*, 2006). However, the nutritional requirements may be influenced by climate change. For example, in periods with high water temperatures, the risk of cataract development may be reduced by increasing the dietary level of histidine (Waagbø *et al.*, 2010; Remø *et al.*, 2014; Sambraus *et al.*, 2017). The dietary lipid concentration in grower feed was increased from 10% in the 1970s to over 35% today, resulting

in a higher need for selected micronutrients in the feed to protect against oxidation (Waagbø, 2008). In addition, high temperature influences the requirements for other micronutrients that are essential to maintain energy metabolism, skin and bone health. As discussed above, elevated temperatures clearly affect bone tissues through physiological, endocrine, nutritional and metabolic mechanisms that could end in bone disorders (Waagbø, 2008). There are, however, measures that may improve the bone health and fish welfare by diet modifications like surplus phosphorus and vitamins C, K and D.

6.6.1 Changes in nutrient requirements

Single nutrient deficiencies may cause clinical disease in fish larvae and juveniles (Tacon, 1993; Halver, 2002; Bæverfjord *et al.*, 2008; Waagbø, 2010; Moren *et al.*, 2011). Most nutrient requirement studies have been performed on juveniles; surprisingly little information is available on nutrient deficiencies or suboptimal nutrition in adult fish, despite this representing the main economical investment and seafood produce (Hardy, 2001). Fish farmers are often faced with similar clinical disease in adults, including variable susceptibility to infectious diseases, suggesting that feeding and nutrition may be important factors in diseases of multifactorial origin (Waagbø, 2006). It is clearly demonstrated that Atlantic salmon may develop organ-specific signs, such as cataract, bone deformities, skin disorders and immunodeficiency related to suboptimal nutrient supplies during intensive commercial aquaculture production.

For farmed Atlantic salmon, risk areas that have been identified where there may be suboptimal micronutrient supplies are: (i) productivity (growth and nutrient utilization); (ii) quality of the final product (for human consumption); and (iii) welfare of the animals. Variable environmental conditions and dietary supplies of B vitamins interfere with the intermediary metabolism of macronutrients, where each of these vitamins have their target biochemical actions (Waagbø *et al.*, 2001). Single B vitamins are needed for the energy extraction from the macronutrients and thereby growth, type of growth (partitioning of the macronutrients) and overall cellular and organ function and development. Organs differ in their intensity of metabolism, supporting aerobic (liver, red muscle, gills and others) or anaerobic oxidation (white muscle). Consequently, these organs may be sensitive to changes in the environment, such

as ambient temperature, and B-vitamin cofactors needed to support the metabolism. From our previous study (Olsvik *et al.*, 2013b), we identified pantothenic acid as a first limiting vitamin in rapidly growing rainbow trout, and this was confirmed histopathologically as ‘clubbed gill filaments’ in deficient trout.

In a preliminary study on the influence of environmental temperatures (4°C, 10°C, 15°C and 20°C) on B vitamin status in Atlantic salmon smolt fed a commercial feed for 60 days, liver and white muscle tissue showed that the status of several vitamins fluctuated greatly and differently (R. Waagbø, 2000, unpublished data; Table 6.1). The observed vitamin status was also below the status observed in vitamin requirement studies (Waagbø, 2010; NRC, 2011; Hansen *et al.*, 2015; Hemre *et al.*, 2016). Table 6.1 indicates the differences in vitamin status in salmon reared at 10°C versus 20°C, which may appear from changes in feed intake, change from aerobic to more anaerobic metabolism, as well as redistribution of vitamins between organs according to the needs. B vitamins act as coenzymes in vital energy-generating processes and changes in overall metabolism (oxidation versus growth) in response to environmental temperature may therefore affect the requirement of the individual B vitamins differently. Recently, practical B vitamin requirements were revised in plant-based diets for fast-growing Atlantic salmon parr in fresh water and for post-smolts in seawater (Hemre *et al.*, 2016), indicating elevated requirements for all the B vitamins relative to the recommendations of the National Research Council (NRC, 2011), except for thiamine and biotin.

6.7 Conclusions

Climate change will affect farming conditions for fish species in aquaculture. One may expect elevated requirements for nutrients, especially within antioxidation, nutrients supporting metabolism, and nutrients that have the potential to alleviate observed production related disorders (e.g. cataracts, bone deformities, skin disorders, gill disorders and reduced immunity). With increased knowledge on the mechanisms on how single and mixed environmental factors affect fish welfare and health it is reasonable to expect that we can meet the challenges with climate friendly feeds that are nutritionally balanced.

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Behaviour including Fish Migration

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7.1 Introduction

The effects of climate change on fish are often examined at the levels of populations and species, but these ultimate consequences are driven by the responses of individual animals to biotic and abiotic stimuli. Determining how individuals cope with changing environmental stimuli requires tools and techniques to evaluate and interpret animal status and their reactions, which may be adaptive or maladaptive. Behaviour plays a critical role in this response, because it moderates the interactions between animal physiology and environmental conditions (Sih *et al.*, 2011; Cooke *et al.*, 2013). Indeed, behaviour is the observable manifestation of cellular and biochemical processes in organisms that in turn influences physiological state, for example by moderating exposure to stressors or the acquisition and expenditure of energy to survive, grow and reproduce. Animals respond to environmental cues by integrating them through sensory systems, releasing hormones and neurotransmitters, and behaving in ways ostensibly aimed at restoring or maintaining homeostasis. This behaviour may be evading a threat or moving away from a point-source stressor such as pollution or degraded habitat (Schreck *et al.*, 1997). This example is best conceptualized by the decision either to move or to remain, when an individual must decide whether to expend energy to find better habitat or remain in the same location aspiring to compensate for the disturbance (e.g. Fig. 7.1). Behaviours can vary widely among individuals in response to the same stimulus but can also provide relevant information about how populations respond to cues (Zala and Penn, 2004; Tuomainen and Candolin, 2011).

Animal behaviour is generally predicted to optimize the trade-offs between maximizing fitness via net energy gain and acquiring mates versus avoiding predators and stressors, with strategies informed by the detection of environmental cues (Pyke *et al.*, 1977; Houston *et al.*, 1993). Behaviours may occur across relatively fine spatial scales (e.g. evading a predator, selecting a food item, or avoiding cold water), or over much larger scales (e.g. dispersal between habitats to find food or mates, migration or cyclic movement between distant areas). Individual strategies including foraging behaviours, diel activity patterns and reproductive frequency are affected by the capacity of the sensory system to integrate environmental stimuli, the potential of the endocrine system to respond to stimuli, and the power of the locomotor system to process energy into action (Dickinson *et al.*, 2000; Nathan *et al.*, 2008). Physiological energy processing is controlled by environmental conditions and therefore intrinsically influenced by climate change in ectotherms (Brown *et al.*, 2004; Chown *et al.*, 2010). Behavioural deficits or disturbances can result in critical changes to energy processing, especially accelerated energy depletion, poor nutrition due to unsuccessful foraging, competitive inferiority, increased vulnerability to predators, or reduced reproductive output owing to ineffective or skipped reproductive attempts (Caro, 1999; Buchholz, 2007; Horodysky *et al.*, 2016).

Physical and chemical changes to water quality and quantity related to climate change may require evolutionary adaptations or behavioural plasticity for individuals to cope and populations to persist (Chown *et al.*, 2010). Contexts may also shift for species as assemblages change as a result of the

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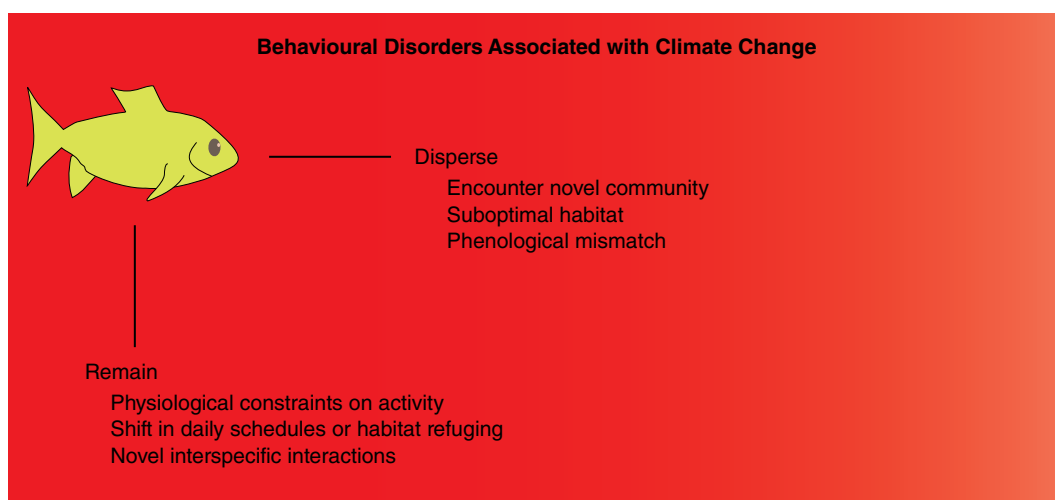


Fig. 7.1. Hypothetical example of a fish confronted by changing environmental conditions (red gradient in the background colour of the figure which changes from red in bottom left to a paler shade in top right). The fish may remain within its normal range where the climate is changing or it may disperse. The possible negative consequences of each of these decisions are listed. Incorrect decisions are suggestive of behavioural disorders, maladaptive behaviour that challenges an individual's physiology.

introduction of new species or disappearance/subordination of native species. Individuals may be resistant (i.e. through physiological mechanisms) or resilient (see Ross *et al.*, 1985) to environmental changes. Resilience can manifest as increased rates of reproduction or movement to compensate. Individuals may therefore exhibit flexibility in their behaviour (i.e. plasticity), but there are also consistent differences among individuals within populations upon which natural selection can act to shape behaviour across generations (Sih *et al.*, 2012). Reaction norms can also control behaviour as responses to the environment differ across gradients of that environment (Dingemanse *et al.*, 2010), and epigenetic flexibility in behaviour may manifest as a result of parental effects (Miller *et al.*, 2012). Studies are emerging in which activity, movement, foraging, reproduction, and other behaviours are modified in animals in association with climate change (Sih *et al.*, 2011). On a macro-scale, individual dispersal may shift species distributions into areas where conditions are favourable (Perry *et al.*, 2005; Parmesan, 2006; Chen *et al.*, 2011); this is most likely for animals in large, open systems such as oceans and large lakes or in species with high mobility (especially in larval phases). On smaller scales the changes in spatial or temporal distribution may be more nuanced involving

changes in the timing of activities or changes in habitat. When fish behaviour is synchronized with cues that are no longer appropriate (i.e. they have become maladaptive or mismatched), the fish may experience an ecological trap with evolutionary consequences (Schlaepfer *et al.*, 2002; Hale and Swearer, 2016; Hale *et al.*, 2016).

Our present discussion focuses on how climate change can induce behaviour–environment mismatches in fish. We focus on how maladaptive behaviours, or behavioural disorders, may arise in response to climate change including increased water temperature, carbon dioxide and decreased oxygen, flow/volume, and pH in aquatic habitats. We present examples of how behaviour will be impacted by climate change and make connections to how the behaviour can be maladaptive (i.e. mismatched, a ‘behavioural disorder’). We will also discuss the limitations and opportunities for behaviour to be applied in a clinical context, prescribe solutions and evaluate the outcomes of treatments implemented for conservation. Whether a behaviour can, or should, be classified as a disorder is likely to be a matter of interpretation in all but the most extreme circumstances. Maladaptive or sub-optimal behaviours may be elicited predominantly when an individual does not have the necessary information or the ability to make appropriate

decisions, for example when anadromous fish return to fresh water too early and deplete their energy reserves before they spawn.

7.2 Diagnosing Behavioural Disorders Associated with Climate Change

Investigations into how behavioural strategies are, or will be, affected by climate change are needed to diagnose disorders, despite species-level documentation that interspecific interactions are often the proximate driver of climate-induced changes to population dynamics and extirpation (Lynch *et al.*, 2016). Behavioural disorders in fish must be diagnosed relative to baseline information on the natural, expected behaviour of an individual or contrasted against the adaptive response to a particular cue. Monitoring fish using electronic tagging (biologging) can identify broad-scale patterns including size of home range or residency (Cooke *et al.*, 2004). Daily activity and foraging schedules or seasonal shifts in distribution/migration can be observed in electronic tagging data or inferred from other sources (e.g. fishery surveys; Sims *et al.*, 2004). Biologgers can extend observations to identify unique activities in the life of an individual fish (e.g. foraging, copulating; Cooke *et al.*, 2016), paired with direct observations/inferences such as snorkel surveys on spawning grounds, particularly for time series investigations. Other tools, such as stomach content analysis, stable isotope ratios and hormone sampling, can be used to add to our knowledge of behavioural activities or contextualize observations.

Individual behaviour has a genetic basis with reaction norms that can, to some extent, synchronize behaviours to contemporary environmental conditions (Dingemanse *et al.*, 2010). Within populations, there is some variation in context-specific behaviour because the genetic basis to this behaviour also interacts with the environment (Biro *et al.*, 2010; Chapman *et al.*, 2011; Brodersen *et al.*, 2012; Dodson *et al.*, 2013). Consistent differences among individuals are correlated suites of behaviours that are consistent across contexts and are indicative of a personality or coping style (Sih *et al.*, 2012; Harrison *et al.*, 2017), which can lead to significant differences within and among populations in behaviour (e.g. Cote *et al.*, 2010). For example, fish may be relatively 'bold' or 'shy' depending on their resource needs (Biro and Stamps, 2008) and physiology (Koolhaas *et al.*,

1999; Niemelä *et al.*, 2013), and this may correlate with other behaviours and activity levels (Biro *et al.*, 2010). The extent of the effect that environmental variation has on the variation within populations (Niemelä *et al.*, 2013) and the individual is probably conserved within species because multiple phenotypes can have equally high fitness or because different strategies are advantageous given different environmental conditions. For example, boldness may be adaptive when resources are abundant, but the metabolic demands become maladaptive when resources are scarce, shifting the fitness landscape (e.g. Hulthén *et al.*, 2017). Intraspecific variation in traits such as metabolic rate and cognitive ability (Niemelä *et al.*, 2013) therefore controls population-level resilience to unpredictable environments and can allow for more rapid evolution under selection (Wolf and Weissing, 2012).

Together, we see that observing, or inferring, behaviour of fish can provide information relevant to evaluating individual fitness. Behaviour can be adaptive or maladaptive and linking changes in behavioural variation or personalities to outcomes when confronted with a climate change scenario is nuanced without long-term study. We will present observations of fish behaviour, including activity, interactions with conspecifics or heterospecifics, and phenology, in the context of climate change and discuss how changes to behaviour, or the lack of it, may become mismatched in a changing environment.

7.3 Field and/or Experimental Studies including Projection(s) of Future Trends with Climate Change

7.3.1 Activity

Daily schedules of fish are synchronized with their energetic needs, which are determined by internal and external factors to acquire/expend energy (Fry, 1971). Many fish species (and individuals; see Biro *et al.*, 2010) are adapted, both behaviourally and physiologically, to the thermal regime of their environment. For example, sockeye salmon (*Oncorhynchus nerka*) are adapted to perform aerobic exercise optimally based on the thermal regime of their natal spawning river and temperature changes may increase reliance on taxing anaerobic pathways (Eliason *et al.*, 2011). Confronted with low water or high temperature, individuals may exhibit daily movements or increased feeding within a system to compensate

for changes to metabolic needs (Como *et al.*, 2014). Increased temperature or acidity or decreased dissolved oxygen results in habitat compression, a phenomenon in which the amount of suitable habitat shrinks, forcing individuals into smaller spaces (Prince and Goodyear, 2006). Consequently, range shifting or daily migrations to thermal refuges may emerge in species impacted by climate change (e.g. warming; Harrison *et al.*, 2016). Individuals may be able to find such refuges from long distances to outlast ephemeral conditions such as temperature spikes (Dugdale *et al.*, 2016). Ambient temperatures above 24°C cue juvenile Atlantic salmon (*Salmo salar*) parr to abandon their territorial behaviour and seek colder thermal refuges to avoid or alleviate heat stress (Breau *et al.*, 2011). However, this refuging behaviour may represent an ecological trap because habitat compression can increase vulnerability to predators by aggregating fish, as in mesopelagic fish pushed shallower into the photic zone as the oxygen minimum layer rises in the ocean (Koslow *et al.*, 2011). Recent experimental evidence demonstrating that heat-stressed salmon use the social, chemical cues of aggregated conspecifics to locate thermal refugia (Elvidge *et al.*, 2017) raise the further complication of these cues being degraded under certain conditions of water chemistry, such as acidity (Leduc *et al.*, 2006; Munday *et al.*, 2010), acting as a secondary stressor (Fig. 7.2).

Temperature (Biro *et al.*, 2010) and pCO₂ (partial pressure of CO₂) (Munday *et al.*, 2010; Cripps *et al.*, 2011; Hasler *et al.*, 2016) can both alter fish behaviours. Munday *et al.* (2010) observed higher vulnerability to predators following exposure to hypercapnic water; however, Regan *et al.* (2016) demonstrated that acclimatization of striped catfish (*Pangasianodon hypophthalmus*), an endangered siluriform of the Mekong River, to acidic fresh water resulted in a reduction in activity attributed to changes in GABA_A (γ-aminobutyric acid, type A) receptor activity. These opposing results emphasize the need for species-specific studies and also illustrate how communities can be changed when competitive balances are disrupted.

Foraging schedules and behaviour are often plastic and respond to changes in biotic or abiotic factors (Godin, 1981; Lall and Tibbetts, 2009). Changes to foraging may signal an attempt to improve conditions or simply avoid malnutrition. Increased daily temperatures alter the daily feeding regimes in stream fish (Fraser *et al.*, 1993) and

marine reef grazers have reduced preference for algae in acidic water (Borell *et al.*, 2013), suggesting changes in physiology or behaviour to select different prey. In marine environments, bonefish (*Albula vulpes*) selectively utilize shallow nearshore foraging habitats at temperatures that are optimal for their aerobic scope to exercise and digest prey, with potential for small-scale distributional shifts away from these habitats as they depart from tolerable temperatures (Brownscombe *et al.*, 2017). Such plasticity may be an effective means of compensating for adverse conditions but in the long-term may impact fish nutrition.

7.3.2 Seasonality

Seasonal schedules allow fish to match foraging, dispersing/migrating and breeding to optimal environmental conditions. Mismatched timing of key events has been observed in many taxa including fish; this phenological phenomenon is observed when animals use environmental cues that no longer synchronize with favourable conditions, and so the behaviour can be rendered maladaptive (Pankhurst and Porter, 2003; Bradshaw and Holzapfel, 2008; Pistevo *et al.*, 2017). Information from altered temperatures driving phenological mismatches have manifested as contaminant bioaccumulation and changes to habitat use, demographic structure, geographical range limits, and community interactions of fish (Otero *et al.*, 2014; Asch, 2015; Guzzo and Blanchfield, 2016; van Walraven *et al.*, 2017).

For many species, dispersal and migration are optional; for example personality can influence dispersal or residency of many species (Cote *et al.*, 2010). Moreover, partial migration is ubiquitous in fish and many have the capacity to make long-distance movements when cued by endogenous or environmental conditions. Brodersen *et al.* (2011) showed that temperature in a lake influenced the seasonal presence of partially migratory roach (*Rutilus rutilus*). More field data are needed to determine whether climate change alters migratory patterns in fish and how they may shift their distributions in response, but in general, the decision to remain or disperse can result in a behavioural disorder when environmental cues create an ecological trap (Fig. 7.1).

As temperatures shift, discord between contemporary and historical photoperiod length and temperature relationships may result in phenological

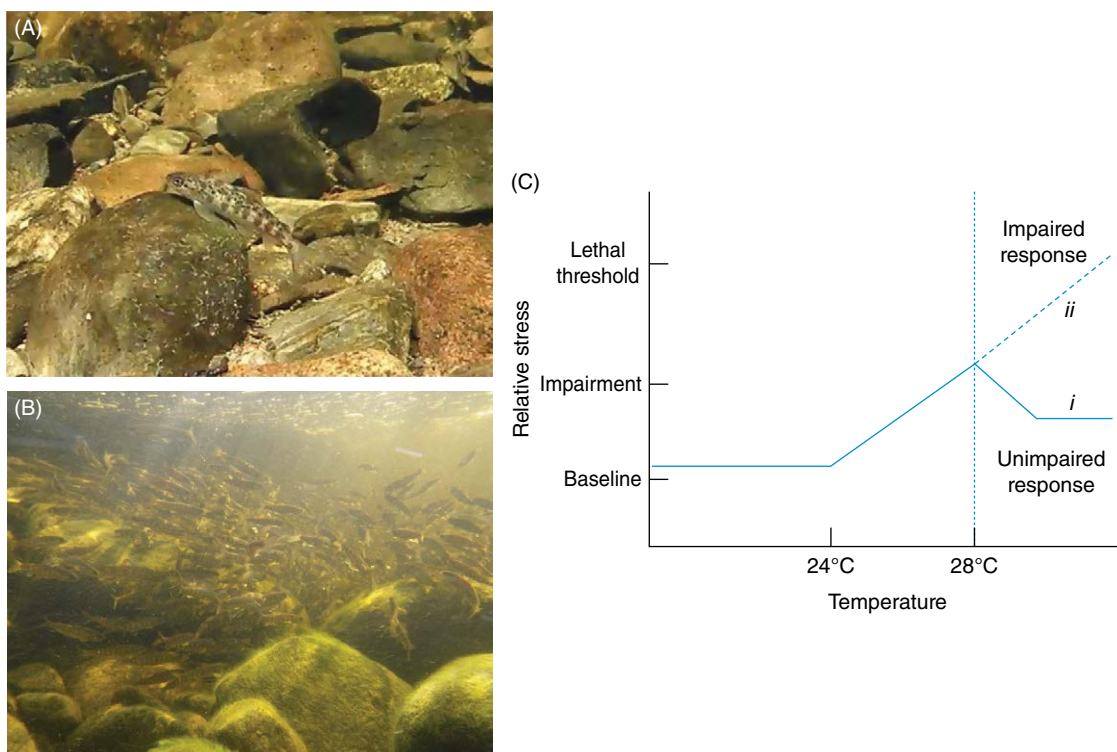


Fig. 7.2. Juvenile Atlantic salmon (*Salmo salar*) are typically aggressive towards conspecifics and defend territories where they forage on invertebrate drift (A). At elevated water temperatures (> 24°C), heat stressed 1+ and 2+ salmon parr abandon this behaviour and leave their territories in search of cold water refugia, where they will aggregate at high densities until water temperatures decrease. One such aggregation (B) was observed in the Little Southwest Miramichi River, New Brunswick, Canada, on 3 July 2010, when water temperature in the mainstem river reached 30.66°C. (C) Heat-stressed juvenile salmonids seeking thermal refugia use the chemical cues of aggregated conspecifics to locate suitable cold-water plumes and reduce their relative physiological stress levels to below lethal thresholds (scenario *i*). Altered water properties (e.g. acidification, chemical pollution, increased salinity) can interfere with fish olfaction and reduce the effectiveness of relying on chemical information to locate thermal refugia (scenario *ii*), precluding a return to sublethal stress levels. This represents an environmentally-mediated behavioural impairment that could result in high mortality rates in populations of salmon and other fishes that are susceptible to heat stress in a changing climate. (A and B courtesy of C.K. Elvidge and R.A. Cunjak, respectively, and reproduced from Elvidge *et al.*, 2017; C by authors of the current chapter.)

mismatches. For example, stickleback (*Gasterosteus aculeatus*) in Arctic lakes breed earlier and more frequently in warmer conditions (Hovel *et al.*, 2017). Some pre-spawning Pacific salmonids (*Oncorhynchus* spp.) may also migrate early into fresh water, only to encounter supraphysiological temperatures that greatly reduce the success of migration (Farrell *et al.*, 2008; Baisez *et al.*, 2011). The timing of entry into fresh water is an evolved behaviour that confers advantages to individuals in terms of fitness but with climate change the fitness landscape is shifting (Katinic *et al.*, 2015; Quinn *et al.*, 2015).

Similarly, Sims *et al.* (2004) modelled fishery catch data to demonstrate how the timing of European flounder (*Platichthys flesus*) migration fluctuates with water temperature. Spatial, rather than temporal, changes to fish populations are also anticipated and some marine species move deeper to more suitable thermal habitat as the ocean warms (Dulvy *et al.*, 2008). Most of these changes have been studied using population means and not individuals, so we know little of how individual behavioural variation and strategies contribute to these potentially adaptive responses.

7.3.3 Interspecific interactions

Physicochemical changes to habitat alter metabolism and influence the decision making of animals that can affect cooperation, competition and predator vulnerability. In many species, intraspecific cooperation is critical to predator defence and there is evidence to suggest that schooling behaviour is negatively affected by hypoxia and elevated temperature, both signatures of climate change that will decrease cooperation and increase vulnerability to predation (Bartolini *et al.*, 2015; Domenici *et al.*, 2017). Dissolution of schools could also affect nutritional status of individuals as they struggle to locate and acquire enough food alone (Pitcher *et al.*, 1982). Interestingly, Hovel *et al.* (2017) observed increased densities and competition of age-0 three-spine stickleback (*G. aculeatus*) in warmer water that reduced the growth rate of these young fish.

The latent impacts of behavioural disorders arising from climate change may be changes to competition and predation that alter fish assemblages (Taniguchi *et al.*, 1998) or distributions within and among systems (Dugdale *et al.*, 2016; Corey *et al.*, 2017). Indeed, fish alter their behaviour in response to temperature increases, which can mismatch foraging efforts with intake needs (Nakayma *et al.*, 2016). These changes in turn may alter energetic intake by spatially or temporally disconnecting fish from their prey items or can result in changes to the dominance ranks within communities if increased temperatures release species from metabolic subordination (Taniguchi *et al.*, 1998; Carmona-Catot *et al.*, 2013).

Behavioural changes at small scales can yield large impacts on fish fitness. Domenici *et al.* (2011) observed a significant decrease in behavioural lateralization of demoiselle damselfish (*Neopomacentrus azysron*) when exposed to high CO₂. These findings were reflected in a similar study using the euryhaline three-spine stickleback (*G. aculeatus*), where freshwater populations responded to acidification with decreased lateralization as well as decreased curiosity and prolonged escape times (Jutfelt *et al.*, 2013). Poor predator escape responses could then impact other aspects of antipredator behaviour. Further research is needed to determine how this might manifest, for example whether impacted fish would seek more cover or alter daily schedules to compensate, and corresponding descriptions of whether it results in behavioural disorders per se. Marine populations of stickleback

also decreased lateralization under acidification, but importantly this did not affect vulnerability to predation (Näslund *et al.*, 2015).

7.4 Control(s) and/or Prevention(s)

Fish respond to cues in their environments. Maladaptive behaviours caused by environmental changes are difficult to prevent because behaviour itself is the response of an individual to some challenge, and the observable manifestation of an attempt to restore physiological imbalances detected through environmental cues (Crozier and Hutchings, 2014). However, there are instances in which the behaviour of an individual may be erroneous and maladaptive because it mismatches with the environment. The timing of migration into fresh water is a behaviour that should confer advantages to individuals in terms of fitness but with climate change the fitness landscape is shifting and the relative fitness may also change (Katinic *et al.*, 2015). To the extent that interactions with other cues can be minimized, individual behaviours can then be expressed to maximize responses to unavoidable climatic change (Lynch *et al.*, 2016).

To ensure the protection of diverse phenotypes to maintain plastic and evolutionary responses, fishery managers should strive to maintain variable genotypes, life history strategies, and available habitats in local waters. For freshwater fish, winter strategies often determine species persistence, but when adverse winter conditions are ameliorated by climate change, formerly adaptive behavioural strategies may no longer be functional, including foraging behaviour, thermal habitat use, predator/prey responses, mate selection, and spawning location (see Shuter *et al.*, 2012). Ultimately, changes in local conditions may release other species from competition and result in shifts in relative abundance and community structure (e.g. Taniguchi *et al.*, 1998).

7.5 Conclusions with Suggestions for Future Studies

Behavioural metrics have the potential to be widely applied to investigate how fish are affected by climate change. We have identified examples demonstrating that climate change alters fish behaviour at various scales, yet it is often difficult to determine whether those behavioural responses are beneficial or harmful to an organism, particularly when

investigated across short timescales (Merilä and Hendry, 2014). Moreover, the underlying genetics and the relationship between genes and environment is still an active field of research (Ellegren and Sheldon, 2008). Behaviour is often plastic within individuals, but the degree of plasticity is variable and is also subjected to selection. In partially migrant species, plasticity in the expression of both the migratory phenotype and the timing of migration is the result of interactions among a variety of factors, including the energetic state of an individual (Halttunen *et al.*, 2013; Peiman *et al.*, 2017). Whereas some behavioural responses to climate change may be maladaptive, variation in the responses is crucial to resilience of populations because it accelerates the rate at which populations may adapt by natural selection (Wolf and Weissing, 2012).

Although phenotypic plasticity and reaction norms may mitigate the effects of environmental changes that are small in magnitude or ephemeral in timing, persistent and extreme changes are more likely to cause intergenerational changes through selection (Bradshaw and Holzapfel, 2008). For example, fish encountering suboptimal temperature or flow en route to spawning grounds may exhibit a phenotypically plastic response (e.g. delay); however, the extent of this plasticity (i.e. slope of the reaction norm) is genetic, and fish that cannot express such plasticity will have reduced fitness. The relative contribution of genetics to adaptive, or maladaptive, behavioural responses is not well established in most cases. This highlights the need for more research into mechanisms driving behavioural changes associated with climate change (Anderson *et al.*, 2013; Munday *et al.*, 2013; Crozier and Hutchings, 2014; Grether, 2014; Merilä and Hendry, 2014). The flexibility that fish have in expressing their behavioural phenotypes may be one key mechanism by which animals can respond to climate change (Tuomainen and Candolin, 2011) and may not be equivalent among populations. Transgenerational acclimatization, where the environment experienced by the parent exerts non-genetic recovery effects on the phenotype of the offspring, may help populations to persist under changing climate conditions (Miller *et al.*, 2012; Munday, 2014).

Future research should focus on life history stages, trade-offs between stages, the duration of any fitness effects, and how epigenetic effects interact with genetic changes (Munday, 2014), in addition to how interactions among climatic variables affect different behavioural phenotypes (Frost

et al., 2013) and cognitive performance (Niemelä *et al.*, 2013; Maille and Schradin, 2017). These behavioural effects should be integrated into more empirical and modelling studies (Koenigstein *et al.*, 2016), especially as they may interact with fisheries-induced changes (Peer and Miller, 2014) because long-term investigation is often needed to properly quantify fitness and identify adaptive versus maladaptive strategies. Further consideration must be given to species in an ecosystem context because changing conditions operate equally, but differently among species and the interspecific and intraspecific interactions are likely to change with potentially unpredictable consequences as assemblages change and dominance shifts. The interplay between behaviour and physiology, including feedbacks within and between these trait categories, is an area of active research, as is the interplay between photoperiodism, changes in physicochemical tolerance, and behaviour in regulating scheduling of life history events (Bradshaw and Holzapfel, 2008; Anderson *et al.*, 2013). Ultimately, the effects of climate change on fish behaviour require further studies including the discovery and mitigation of behavioural mismatches and disorders.

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8

Stress in Response to Environmental Changes

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8.1 Introduction

The physiological responses elicited by animals to changes in their environment are essential for survival and often an indicator of organismal fitness (Breuner *et al.*, 2008; Schreck and Tort, 2016). As species undergo physiological adjustments in response to climate change, it is increasingly important to examine the endocrine factors that may play a role in adapting (or maladapting) animals to the changing environment (Fig. 8.1). The corticosteroid-stress response, and the resulting physiological alterations that allow animals to cope with stress have been well characterized in fishes (Wendelaar Bonga, 1997; Mommsen *et al.*, 1999). However, changes in abiotic variables associated with climate change, and its influence on the stress response is far from clear. Here we will focus on environmental factors that are affected by climate changes in influencing the endocrine stress response. It has also recently been reported that the exposure of parents to environmental changes may improve progeny survival in fish, implicating epigenetics as a mechanism for long-term and generational adjustments to environmental stressors (Ryu *et al.*, 2018). Environmental impacts on epigenetics in teleosts has been recently reviewed (Best *et al.*, 2018), and are outside the scope of this chapter.

The concept of stress response in animals, including fish, can be broadly divided into three categories, the primary, secondary and tertiary responses (Fig. 8.1) (Mazeaud *et al.*, 1977; Barton, 2002; Schreck and Tort, 2016). The primary response is the initial neuroendocrine response to the perceived

stressor. It is characterized by the release of hormones, including catecholamines and corticosteroids, of which cortisol, the principle corticosteroid in teleosts, is predominantly used as the marker of the primary stress response (Vijayan *et al.*, 2010; Schreck and Tort, 2016). These hormonal responses, either singly and/or in combination elicit a series of secondary physiological responses, including energy substrate mobilization, ion disturbances and immune function, that dictate the extent to which an animal can restore homeostasis or survive with a newly established allostatic load (McEwen, 2005; Gorissen and Flik, 2016; Schreck and Tort, 2016). It is here where environmental changes, such as temperature will have the biggest impact in fish. As poikilotherms, they are subject to the changes in environmental temperature, which can impact both resting metabolism and the metabolic readjustments in response to stress. Nutrient homeostasis and efficient energy substrate reallocation are the driving forces that govern how well the animal copes with stress, as well as the longer-term fitness of the animal (Mommsen *et al.*, 1999; Faught *et al.*, 2016). Both the primary and the secondary responses are observed over an acute stressor period and, especially, the secondary responses may range from minutes to hours to days (Mazeaud *et al.*, 1977; Wendelaar Bonga, 1997; Barton *et al.*, 2002). The tertiary stress response is population-level changes that result when an animal fails to properly cope with the increased energy demands. End points of the tertiary stress response include a negative growth rate, increased disease susceptibility and

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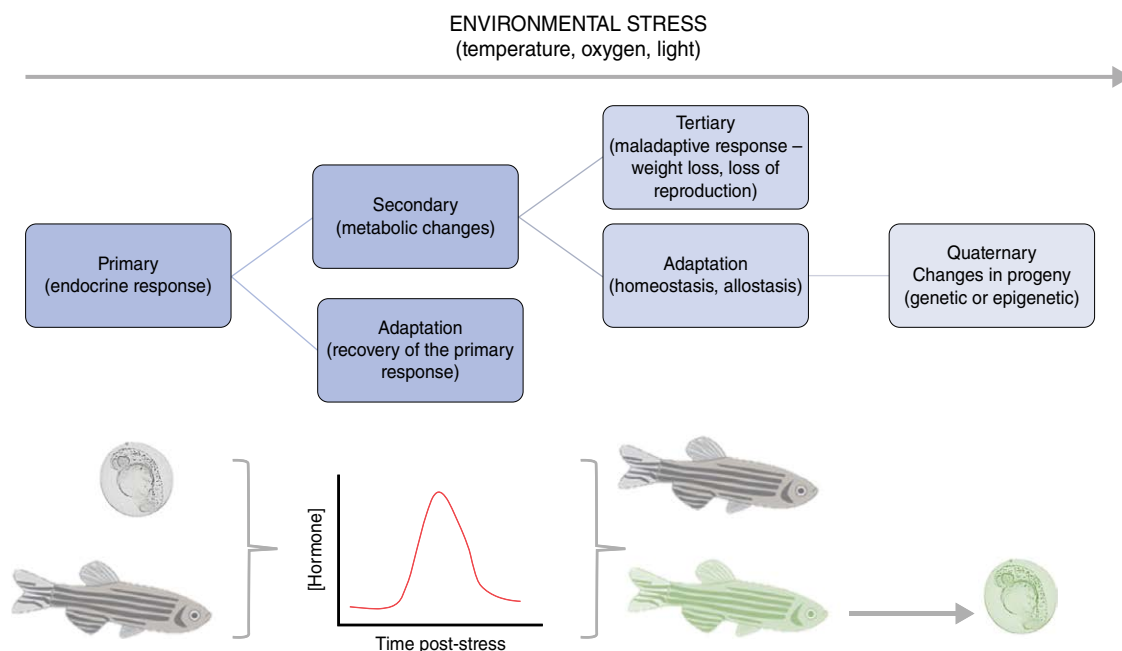


Fig. 8.1. The effect of environmental stress on the stress response in fish. Changes in abiotic factors such as temperature, oxygen and light can act as environmental stressors which will impact the stress response of fish. The primary stress response is characterized by an increase in corticosteroids and catecholamines. The secondary response to stress is due to signalling by these hormones to increase energy availability by enhancing glycogenolysis (glucose), lipolysis (free fatty acids) and proteolysis (amino acids). Negative feedback will also restore resting levels of these hormones under acute stressor conditions. The tertiary stress response describes a maladaptive response to stress in which the fish will lose weight and reproductive function will be impacted. Conversely, the fish can also reach an allostatic state (green fish), whereby they have adapted to live in the presence of a stressor, and this may be passed on to the next generation by genetic and/or epigenetic changes (green embryo) which may alter the progeny's response (the quaternary stress response) to environmental stressors.

poor reproductive performances, all of which reduce fitness (Mazeaud *et al.*, 1977; Schreck and Tort, 2016). We also will introduce the concept of the quaternary stress response as a fourth response to stress, which describes the transfer of genetic and epigenetic factors from parents to progeny, as a means to adapt to the changing environmental stressors (see Section 8.5). Although no study has specifically examined the impact of climate change on the stress responses (Conde-Sieira *et al.*, 2018; Vargas-Chacoff *et al.*, 2018), rising water temperature, and the associated reduction in oxygen diffusion, may compound the metabolic demands on fishes. Consequently, we limit the scope of this chapter to the primary and secondary metabolic stress responses, as the energy substrate dynamics are a primary determinant for how the fish copes with stress (Faught *et al.*, 2016).

8.2 The Primary Stress Response

The first step in stressor-sensing is the activation of neuroendocrine responses with the immediate result of increasing energy substrate availability to cope with the energy demand that accompanies stressor exposure (Wendelaar Bonga, 1997; Mommsen *et al.*, 1999). This rapid neuroendocrine response encompasses the simultaneous activation of two key axes: (i) the sympathetic nervous system–chromaffin cell (SNC) axis, which results in the release of stored epinephrine and norepinephrine within seconds to minutes of sensing the stressor (Wendelaar Bonga, 1997; Vijayan *et al.*, 2010); and (ii) the hypothalamus–pituitary–interrenal (HPI) axis, which results in the synthesis and release of cortisol, the primary glucocorticoid in teleosts (Fig. 8.2) (Wendelaar Bonga, 1997; Mommsen *et al.*, 1999). The activation of the SNC axis is rapid, and the catecholamine release,

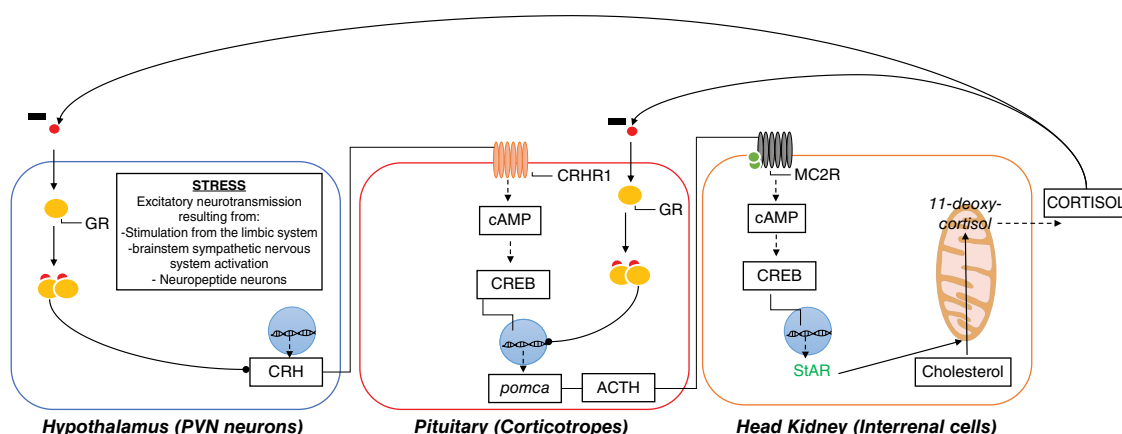


Fig. 8.2. Hypothalamus–pituitary–interrenal (HPI) axis activation and biosynthesis of cortisol. Stress will cause release of corticotropin releasing hormone (CRH) from the hypothalamus, which stimulates the corticotropes of the pituitary to release adrenocorticotrophic hormone (ACTH). Binding of ACTH to melanocortin-2-receptor (MC2R) of the interrenal cells will stimulate production of cortisol from cholesterol by increasing steroidogenic acute regulatory protein (StAR) transcription. StAR will facilitate the transport of cholesterol across the inner mitochondrial membrane. Cortisol will act through the glucocorticoid receptor (GR) through a negative feedback loop to restrict cortisol levels. cAMP, cyclic adenosine monophosphate; CREB, cAMP response element; CRHR1, CRH receptor 1; MRAP, melanocortin-2-receptor accessory protein; pomca, pro-opiomelanocortin A; PVN, paraventricular nucleus; StAR, steroidogenic acute regulatory protein.

predominantly epinephrine, in response to stressor exposure occurs within seconds to minutes and they are cleared rapidly, within minutes, from the circulation (Reid *et al.*, 1998). This is one reason why plasma epinephrine release cannot be used as a reliable marker of acute stress response in fishes because of the difficulty of obtaining resting levels of these amine hormones (Vijayan *et al.*, 2010). The majority of the circulating epinephrine in response to stressor exposure is produced by the chromaffin cells, which are distributed along the post cardinal vein in the head kidney of fishes (Reid *et al.*, 1998). The mode of action, leading to catecholamine synthesis and release has been discussed in detail previously (Fabbri *et al.*, 1995; Reid *et al.*, 1998; Fabbri and Moon, 2015). Briefly, cholinergic stimulation by the nicotinic and muscarinic receptors plays a role in the calcium-mediated exocytosis of epinephrine from chromaffin cells (Reid *et al.*, 1998). In addition to cholinergic stimulation, several other hormones, including cortisol and adrenocorticotrophic hormone (ACTH) modulate catecholamine release from chromaffin cells highlighting the multifactorial control of this amine release during stress in fish (Reid *et al.*, 1998).

The HPI axis activation and cortisol release lags behind the catecholamine response and, therefore, quickly anesthetizing the fish upon capture provides a

means to obtain the reliable resting levels of this steroid (Iwama *et al.*, 1998). The HPI axis activation commences with the stimulation of corticotropin releasing factor (CRF) from the hypothalamus, and this peptide in turn stimulates the release of ACTH from the pituitary gland (Wendelaar Bonga, 1997; Faught *et al.*, 2016; Gorissen and Flik, 2016). Studies have shown that both CRF and CRF binding-protein (CRFBP) are regulated by stressors, including cortisol. While we can hypothesize that this may be under the transcriptional control of corticosteroid receptors, the exact mechanisms are unclear in teleosts (Bernier and Peter, 2001; Alderman and Bernier, 2009; Faught and Vijayan, 2018a). CRF is found primarily in the parvocellular neurons of the paraventricular nucleus of mammals and in the preoptic area of teleost fish (Backström and Winberg, 2013). Several neurotransmitters and neuromodulators, including monoamines are thought to regulate the perception of stressors leading to hypothalamus release of CRF (Winberg and Nilsson, 1993). In fish, urocortin (UI) and CRF can also be released from the caudal neurosecretory system (CNSS). While there has been no physiological role for CNSS release of UI or CRF in the stress response, it is thought the CNSS may be involved in osmoregulation (Craig *et al.*, 2005). The scarcity of antibodies against species-specific CRF often precludes measuring hormone levels in fishes; however, CRF transcript levels are upregulated

in response to stressors and correlate with elevated cortisol levels during stress (Bernier *et al.*, 2004; Doyon *et al.*, 2006; Nesan and Vijayan, 2016). This suggests that CRF is not only the primary signal for HPI axis activation, but is also under transcriptional control of both rapid secondary signalling cascades and corticosteroid receptors (Aguilera *et al.*, 2007). CRF binds to CRF receptors on the corticotropes of the anterior pituitary leading to the release of ACTH. Studies have shown higher ACTH positive cells in the corticotropes in response to stress, and a few studies have also measured this peptide in the plasma and it is shown to correlate with the cortisol response during stress (Swift, 1982; Doyon *et al.*, 2006). ACTH is a post-translational product of pro-opiomelanocortin (POMC) synthesis, and as such, this gene is also regulated by other hormones, including corticosteroids (Bumaschny *et al.*, 2007). Similar to CRF, glucocorticoid response elements (GREs) are present in the promoter region of POMC. Excess cortisol stimulation causes a reduction in the transcript abundance of POMC, thereby regulating the steroid levels through a negative feedback loop to re-establish homeostasis (Bumaschny *et al.*, 2007). However, studies on the regulation of CRF and ACTH release – that is the HP regulation – lags behind studies on the interrenal regulation of cortisol release. This is partially due to the ease of measuring factors controlling cortisol release using *ex vivo* and *in vitro* techniques (Patiño *et al.*, 1987; Young, 1988; Bradford *et al.*, 1992; Leblond and Hontela, 1999; Aluru and Vijayan, 2006, 2008; Miller and Hontela, 2011; Sandhu and Vijayan, 2011).

The release of ACTH from the pituitary gland into the circulation stimulates the melanocortin-2-receptor (MC2R) on the steroidogenic cells distributed in the head kidney region of teleost fish (Aluru and Vijayan, 2008). MC2R is a G-protein coupled receptor, which activates adenylate cyclase and increases intracellular cyclic adenosine monophosphate (cAMP) levels. The subsequent phosphorylation/activation of cAMP-dependant protein kinase (PKA) stimulates CREB (cAMP response element-binding) to transcribe steroidogenic acute regulatory protein (StAR) (De Jousineau *et al.*, 2012). Activation of MC2R within the steroidogenic cells of the interrenal tissue is dependent on the accessory proteins MRAP1 and MRAP2. In particular MRAP1 seems to be the most critical for MC2R activation in teleosts, and is involved in the recruitment of MC2R to the membrane (Dores *et al.*, 2014; Sandhu *et al.*, 2019). However, MRAP2 was also shown to be an important component for the functional expression of MC2R in sea bass (*Dicentrarchus labrax*:

Agulleiro *et al.*, 2010; Cerdá-Reverter *et al.*, 2012). Within the steroidogenic cells, StAR is the rate limiting step in steroidogenesis and responsible for the transport of cholesterol across the outer mitochondrial membrane (Stocco, 2001). In humans, the promoter region of StAR has two *cis* elements that are responsible for basal and cAMP-regulated gene expression by steroidogenic factor 1 (SF1) (Sugawara *et al.*, 1997). In teleosts, higher mRNA levels of StAR are correlated with increased stress in fish (Geslin and Auperin, 2004; Aluru and Vijayan, 2006). However, expression of this gene transcript is increased in hypercortisolemic glucocorticoid receptor (GR) knockout zebrafish (*Danio rerio*: Ziv *et al.*, 2013; Faught and Vijayan, 2018a), suggesting that GR may have some negative regulatory control on StAR transcription. Indeed, there are four GREs located 1000 base pairs (bp) upstream of StAR in zebrafish, and in particular, the tandem elements at –136 and –165 may be indicative of a negative GRE (Dostert and Heinzel, 2004).

The regulation of the primary stress response by glucocorticoids occurs through various mechanisms, including modulation of adrenergic receptors of the SNC (Reid *et al.*, 1998), negative feedback of the HPI axis (Alderman *et al.*, 2012; Faught and Vijayan, 2018a), autoregulation of GR (Sathiyaa and Vijayan, 2003), and modulation of 11-βHSD2 (11-β-hydroxysteroid dehydrogenase 2) (Alderman and Vijayan, 2012; Best and Vijayan, 2017; Faught and Vijayan, 2018a). Interestingly, fish that are deficient in GR are hypercortisolemic, which is thought to be not only due to a hyperactivity of the HPI axis (Ziv *et al.*, 2013), but also through a lack of cortisol metabolism (Faught and Vijayan, 2018a). This was confirmed in fish lacking the mineralocorticoid receptor (MR), which had normal cortisol levels (Faught and Vijayan, 2018a), indicating that in addition to HPI activation, the metabolism of cortisol may also contribute to re-establishing the plasma cortisol profile after an acute stress response. While very few studies have examined the effect of environmentally realistic changes in temperature on the primary stress response, the overriding notion is that elevated temperature increases HPI axis activity, leading to higher plasma cortisol levels (Conde-Sieira *et al.*, 2018; Vargas-Chacoff *et al.*, 2018). Temperature effects on the primary stress response may be species specific, as well as dictated to a large extent by the life history and habitat of the animal. For instance, Antarctic fishes (eg. polar cod, *Boreogadus saida*) rarely show a corticosteroid stress response,

and the adrenergic stress response also appears to be muted and species specific compared with the temperate fishes (Whiteley and Egginton, 1999; Whiteley *et al.*, 2006). The implications of climate change in adapting these fishes to secondary stressors, given the higher metabolic demand in warmer waters for these stenothermal animals (Windisch *et al.*, 2014), awaits further study.

8.3 The Secondary Metabolic Stress Response

The metabolic response to stress has been well characterized in fish, and is strongly correlated with the primary stress response described earlier in Section 8.2 (Wendelaar Bonga, 1997; Mommsen *et al.*, 1999; Barton, 2002; Vijayan *et al.*, 2010; Faught *et al.*, 2016). The secondary stress response is essential for re-establishing homeostasis, and the metabolic adjustments involve modulation of energy substrate mobilization, as well as target-tissue energy substrate utilization. Both catecholamines and corticosteroids are responsible for energy substrate mobilization, which primarily includes glucose, lactate and amino acids (Fabbri and Moon, 2015; Faught *et al.*, 2016). Catecholamines are particularly potent regulators of acute hepatic glucose release (Reid *et al.*, 1998; Fabbri and Moon, 2015). Briefly, epinephrine activates beta-adrenoceptors, G-protein coupled receptors (GPCRs), which stimulate rapid glucose release from glycogen via PKA activation (Fabbri and Moon, 2015). Consequently, SNC axis activation increases circulating glucose levels rapidly by glycogenolysis. Although beta-adrenoceptor activation may also lead to gluconeogenesis (Mommsen, 1986; Mommsen *et al.*, 1988), the primary role for this hormone during acute stress adaptation is in glucose regulation by hepatic glycogen breakdown (Fabbri and Moon, 2015). This eventually leads to liver glycogen depletion, an important substrate store for circulating glucose during stress. Catecholamines are also involved in other acute stress-related changes, including heart rate, blood flow, vasodilation (Reid *et al.*, 1998), all geared towards rapid distribution of energy substrates and oxygen for coping with the increased energy demands (reviewed by Fabbri and Moon, 1995, 2015; Reid *et al.*, 1998). A glucocorticoid-driven transcriptional programme is another mechanism of action to increase energy mobilization during stress. GR is a ligand-activated transcription factor, which binds to glucocorticoid

response elements (GREs) in promoter regions of target genes to either activate or repress transcription (Faught *et al.*, 2016). However, despite many studies which correlate the corticosteroid stress response or glucocorticoid treatment with increased enzyme activity, very little is known about the transcriptional regulation of GR on key enzymes involved in energy mobilization.

8.3.1 Glucose as fuel

Glucose is one of the most fundamental energy substrates in all vertebrate metabolism. It can be absorbed through the gut during digestion or produced endogenously by the liver and kidney through either the breakdown of glycogen (glycogenolysis) or *de novo* synthesis (gluconeogenesis) from amino acids and/or glycerol (Mommsen *et al.*, 1999) (Fig. 8.3). The regulation of glucose availability and storage is tissue specific, and both catecholamines and glucocorticoids play key roles in glucose regulation during stress in fish (Mommsen *et al.*, 1999; Fabbri and Moon, 2015; Faught *et al.*, 2016). An increase in plasma glucocorticoids is often correlated with an increase in plasma glucose. While catecholamines are responsible for the initial increase through regulation of hepatic glycogen phosphorylase (GP) (Fabbri *et al.*, 1998), an increase by corticosteroids is also essential for the organism to cope and to recover from a stressor (Mommsen *et al.*, 1999). Indeed, while glucose production 2 h post-confinement in Mozambique tilapia (*Oreochromis mossambicus*) is thought to be primarily due to glycogen breakdown, high glucose 24 h post-stressor is due to gluconeogenesis (Vijayan *et al.*, 1997). Key enzymes involved in liver gluconeogenesis are under the transcriptional control of GR, and can be abolished using a GR antagonist (Sathiyaa and Vijayan, 2003; Vijayan *et al.*, 2003; Aluru *et al.*, 2007). While cortisol treatment has been associated with a decrease in hepatic glycogen *in vitro* and an associated rise in GP in rainbow trout (*Oncorhynchus mykiss*), there is no evidence in teleosts of transcriptional activation by GR in glycogen synthesis/breakdown. This suggests that either we have not yet undertaken the necessary empirical studies, or that cortisol regulation of glycogen breakdown is non-genomic, similar to epinephrine. Indeed, cortisol is capable of activating secondary signalling cascades, including PKA in rainbow trout hepatocytes (Dindia *et al.*, 2012, 2013).

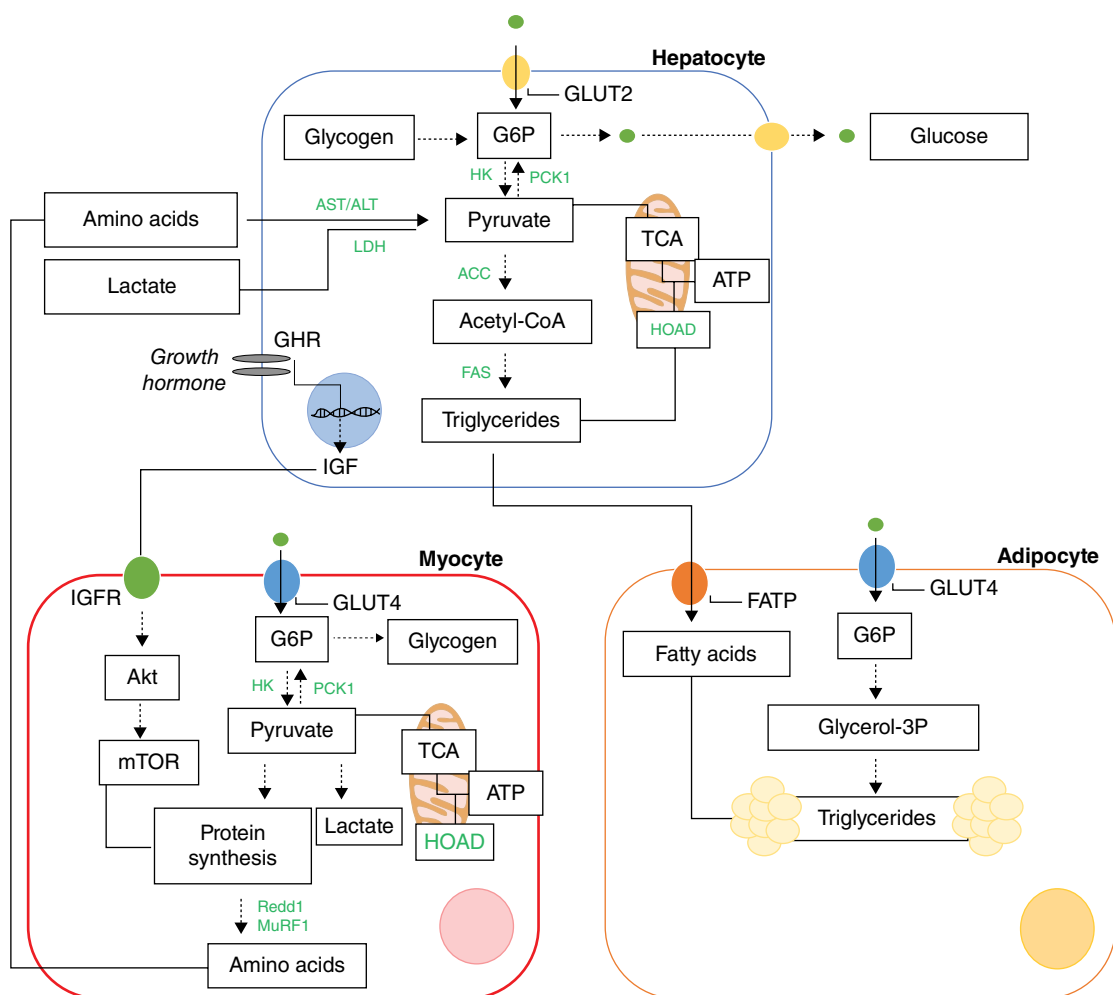


Fig. 8.3. Metabolic regulation during stress. During basal conditions, hepatocytes will take up glucose through glucose transporter 2 (GLUT2) transporters and store it as glycogen. Pyruvate can also be used to produce triglycerides, which contribute to adipose tissue formation. Growth hormone signalling in the liver causes an upregulation of insulin-like growth factor 1 (IGF1) which signals the liver to increase protein synthesis through mammalian target of rapamycin (mTOR). Glucose and fatty acids can be taken up by adipocytes, which increase triglycerides. During stress, hepatocytes will produce glucose through gluconeogenesis, which can be taken up by the muscle through glucose transporter 4 (GLUT4). Cortisol will also stimulate the breakdown of proteins by upregulating E3 protein ligases such as muscle RING-finger protein 1 (MuRF1). This liberates amino acids, which can be used by the hepatocyte to fuel gluconeogenesis. Cortisol will also breakdown triglycerides through β -oxidation, to produce energy (ATP). Enzymes/proteins in green are regulated by cortisol. ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine-triphosphate; FAS, fatty acid synthase; FATP, fatty acid transport protein; G6P, glucose-6-phosphate; GHR, growth hormone receptor; HK, hexokinase; HOAD, 3-hydroxylacyl-CoA; IGFR, IGF1 receptor; LDH, lactate dehydrogenase; PCK1, phosphoenolpyruvate carboxykinase; Redd1, regulated in development and DNA damage responses 1; TCA, tricarboxylic acid cycle.

Classically, cortisol will increase gluconeogenesis by upregulating key genes, such as phosphoenolpyruvate carboxykinase (PCK1/PEPCK). In mammals the transcription of pyruvate carboxylase (PC),

fructose-1,6-bisphosphate 1 (FBP1), phosphofructokinase 1 (PFK1) and glucose-6-phosphate catalytic subunit (G6Pase) and G6P transporter are all under the transcriptional control of glucocorticoids (Kuo

et al., 2015). In fish, the transcriptional control of these genes by GR is not well characterized. Cortisol was correlated with higher mRNA abundance of *pck1* in rainbow trout (Vijayan *et al.*, 2003), and in higher enzyme activity in the hypercortisolic socially subordinate rainbow trout (DiBattista *et al.*, 2006). Some evidence from the rx3 zebrafish mutants (glucocorticoid deficient) also suggests that *pck1* can be rescued by dexamethasone injection and is thus under transcriptional control of GR (Weger *et al.*, 2016). Direct transcriptional control of *pck1* was demonstrated in rainbow trout liver using the GR antagonist RU486 (Aluru *et al.*, 2007), where RU486 abolished the cortisol-induced increase in *pck1* mRNA. Cortisol implants in brook charr (*Salvelinus fontinalis*) were correlated with an increase in PFK1, and G6Pase enzyme activity (Vijayan *et al.*, 1991); however, GR transcriptional regulation of these gluconeogenic enzymes in fish is unclear.

Interestingly in exercised fish, high circulating cortisol levels are correlated with lower rates of muscle glycogenesis (Milligan, 2003). However, the hormonal response may be dictated by the initial glycogen status of the muscle, as lower glycogen content favours glycogenesis by glucocorticoid and epinephrine (Frolow and Milligan, 2004). The rapid glycogenesis in metyrapone-treated rainbow trout (low cortisol) was associated with an increased stimulation of glycogen synthase and a reduction in glycogen phosphorylase activity (Milligan, 2003). This suggests that not only does the skeletal muscle limit glucose, but that it has also adapted to recover glycogen more slowly in the presence of cortisol. It is unknown whether cortisol will impact transcript abundance of glycogen synthase paralogs (*gys1/gys2*) or glycogen phosphorylase (*pygma*, *pygmb* and *pygl*). A working hypothesis at this point is that the restriction in muscle uptake of glucose during stress diverts glucose towards smaller, yet highly aerobic tissues, including the brain. This is supported, in part, by the work of Blasco *et al.* (1996), which clearly showed that the highest rate of glucose uptake was in the brain, while the majority of glucose was taken up by the muscle because of its larger mass (Blasco *et al.*, 1996). It is well known in mammals that glucocorticoids restrict glucose uptake in skeletal muscle and the GR antagonist has been approved for use in type 2 diabetes; however, the mechanism of action is unknown (Bernal-Sore *et al.*, 2018). The restriction of glucose uptake by the muscle was also shown recently in GR knockout zebrafish (Faught and Vijayan, 2019), suggesting an

evolutionary conserved role for GR in glucose reallocation during stress. While most studies have examined the glucose-producing capacity of the stress hormones, we know very little about the target tissue uptake capability, and the fate of the glucose during stress in fish. Understanding the corticosteroid-driven fate of glucose may become important in terms of fuel reallocation and utilization in response to increase metabolic demands due to temperature change.

8.3.2 Energy substrates for oxidation and gluconeogenesis

Skeletal muscle in fish comprises a significant portion (> 50%) of their final body weight (Sadoul and Vijayan, 2016) and is, therefore, an excellent source of energy as stored protein (Mommsen, 2001). Regulation of protein stores in the muscle by glucocorticoids appears to be both catabolic (protein breakdown) and anti-anabolic (inhibition of protein synthesis). In mice and in fish with a GR knockout in the muscle, there was a reduced capacity for protein breakdown, even under high cortisol or fasting conditions (Shimizu *et al.*, 2015). There is strong evidence from mammalian studies that the primary changes in muscle mass in response to cortisol are due to changes in the protein synthetic rate, and that the protein degradation is secondary and adaptive in response to stress (Rennie, *et al.*, 1983). In recent years, work on mammals has highlighted potential targets of GR involved in muscle catabolism. These include muscle specific E3 ubiquitin ligases, muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) (Kuo *et al.*, 2015). Interestingly, both of these genes are important in myofibril integrity in zebrafish cardiomyocytes (Shimizu *et al.*, 2017), but the effect of cortisol on these muscle-specific ligases in fish has not been determined. Classical negative regulators of muscle growth and differentiation, such as myostatin, are also targets of cortisol-GR signalling in mammals, but this may not be the case in salmonid fish. Not only are there no identifiable GREs in the promoter regions of the myostatin orthologs in rainbow trout, but exogenous cortisol treatment failed to induce mRNA transcription in trout myoblasts (Galt *et al.*, 2014). Although the targets of glucocorticoid signalling are not clear in fish, it is clear that cortisol-induced proteolysis is an essential part of the metabolic stress response as amino acids released from the muscle, in response to protein

catabolism, are utilized by the liver for oxidation and gluconeogenesis (Mommsen *et al.*, 1999). This glucose is either shunted into the circulation for use by target tissues to cope with the increased energy demand associated with stress and/or used for replenishing the liver glycogen stores that are depleted due to the initial stressor-induced catecholamine stimulation (Vijayan *et al.*, 1997).

In mammals, cortisol is a potent catabolic hormone that under chronically elevated conditions can lead to muscle wasting (Kettelhut *et al.*, 1988). Gluconeogenic amino acids are provided from muscle catabolism; however, the mechanisms are unclear. Several key targets of muscle breakdown are under transcriptional control of GR (Fig. 8.3). For instance, *Redd1* is a stress response gene that is induced under various stressful conditions, including hypoxia, DNA damage and energy stress. Studies of *Redd1* function in teleosts is limited to developmental studies in zebrafish (Feng *et al.*, 2012). In mice, *Redd1* is a glucocorticoid-responsive gene in the muscle that is thought to reduce muscle metabolism to enable adaptation under energetic stress (Britto *et al.*, 2018). *Redd1* protein is a negative regulator of muscle mass through inhibition of the Akt/mTORC1 signalling pathway and is induced following glucocorticoid secretion. Furthermore, *Redd1* deletion will abolish glucocorticoid-induced skeletal muscle atrophy in mammals (Britto *et al.*, 2014). In fish, genes such as cathepsin D, are under the transcriptional control of GR in the liver, but this remains to be determined in the muscle (Aluru *et al.*, 2007). In zebrafish lacking GR, there is a marked increase in muscle protein content, including higher expression of key translation factors, which supports the postulation that the anti-anabolic effects of cortisol may be mediated by GR in fishes (Faught and Vijayan, 2019).

Apart from glucose and amino acids, lipids and their constituent fatty acids are also major energy sources in fish. Lipids in poikilothermic vertebrates are stored at several sites (mesenteric, liver, muscle) as triacylglycerols and polyunsaturated fatty acids (PUFAs) (Sheridan, 1988, 1994). Lipid storage occurs during periods of feeding, and high insulin levels, while lipid depletion occurs during transitional and non-feeding periods, such as salmonid smoltification (Sheridan, 1988, 1994). Although diet, age and reproductive cycle all play a role in fat deposition, glucocorticoids also play a key role in adipogenesis, but this is poorly understood in teleosts. In mammals, the effect of glucocorticoids on

fat biosynthesis is well known. In diseases with chronically high cortisol levels, such as Cushing's, there is often a layer of fat that is synthesized (Peeke and Chrousos, 1995). Indications of glucocorticoid involvement in adiposity in fish arose when low or high responders to stressors in trout were reared separately. High-responding trout had lower lipid levels when subjected to a crowding stressor compared with either the control or low-responding strains (Trenzado *et al.*, 2006). While a higher degree of lipolysis could be the case in the high-responding trout, genes involved in adipogenesis may also be under glucocorticoid transcriptional control of corticosteroid receptors. Indeed, socially subordinate trout, which exhibit chronically high cortisol levels, have elevated concentrations of circulating free fatty acids and lowered plasma total cholesterol levels (Kostyniuk *et al.*, 2018), supporting that GR activation by cortisol is primarily lipolytic (Mommsen *et al.*, 1999). Interestingly, in the GR knockout zebrafish there is an increase in the amount of fat reported in adults, suggesting that fat deposition may be independent of GR, but still governed by the high cortisol levels in these fish (Facchinello *et al.*, 2017; Faught and Vijayan, 2019).

Studies have shown that stress and/or cortisol increases the activity of lipolytic enzymes in fish (Sheridan, 1988, 1994; Vijayan *et al.*, 1991; Mommsen *et al.*, 1999). Also, adrenergic stimulation (norepinephrine but not epinephrine) showed a lipolytic effect in salmon liver leading to glycerol release *in vitro* (Sheridan, 1988). The glycerol released from the breakdown of triglycerides has the potential to be used as gluconeogenic substrates in fish (Mommsen *et al.*, 1999). Under acute stress, lipolysis can be activated to increase energy demands. The catabolism of fatty acids, β -oxidation, involves the sequential cleavage with acetyl-CoA as a usable end point for the tricarboxylic acid cycle (Tocher, 2003). Various lipases including triglyceride lipase, lipoprotein lipase and lysosomal lipase are responsible for lipolysis in fish (Sheridan, 1988, 1994). While lipoprotein lipase was differentially expressed in rainbow trout liver in response to an acute stressor, a direct role for GR has not been determined (Wiseman *et al.*, 2007). Gilthead sea bream (*Sparus aurata*) held at a high stocking density resulted in high cortisol levels that was correlated with a reduction in liver lipid content and altered lipid composition (Montero *et al.*, 2001). However, high cortisol levels are not always associated with lipolysis, as high cortisol concentration

coupled with a high fat diet led to increased liver lipid deposition, but reduced muscle lipid content (Montero *et al.*, 2001). This suggests that cortisol may act through different mechanisms in a tissue-specific manner to encourage lipid storage to maintain homeostasis. Maintenance of energy homeostasis during food deprivation is directly related to the capacity of the liver to mobilize lipid reserves (Sheridan and Mommsen, 1991). Overall while there is a known link between glucocorticoids and lipid metabolism, the mechanism(s) of action are not well understood in teleost fish.

8.3.3 Role of cortisol in energy substrate repartitioning

Given that stress is energy demanding, a key aspect of the glucocorticoid stress response is thought to be the control of energy substrate partitioning (Mommsen *et al.*, 1999; Faught and Vijayan, 2016; Faught *et al.*, 2016). While glucose regulation occurs at the inter-tissue level, glucocorticoids may also divert intracellular resources to limit energy demanding processes. One example of this is the suppression of immune function. Stress and cortisol have been shown to suppress acute immune response in fishes (Tort, 2011; Schreck and Tort, 2016). This is thought to be an adaptive response to limit the partitioning of energy towards growth and immune homeostasis in order to cope with stress (Schreck and Tort, 2016). It was recently proposed that the suppressors of cytokine signalling (SOCS) may play a key role in regulating the energy substrate reallocation in the liver in response to stress and/or cortisol stimulation (Philip and Vijayan, 2015). The SOCS belong to a family of intracellular proteins, and are important regulators of growth, development and immunity (Kile and Alexander, 2001). They are negative regulators of cytokine signalling and this is mediated largely by inhibiting the JAK/STAT pathway (Kile and Alexander, 2001). As the JAK/STAT signalling pathway is activated by not just cytokines, but also growth hormone, leptin and prolactin, SOCS plays a key role in integrating growth and immune functions (Kile and Alexander, 2001). In teleosts, homologues of all the eight mammalian SOCS family members have been cloned and sequenced, and their role appears to be highly conserved (Wang and Secombes, 2008; Wang *et al.*, 2011). In rainbow trout hepatocytes, cortisol upregulates SOCS mRNA, and this was inhibited by a GR antagonist,

suggesting a direct role for cortisol-GR signalling in SOCS regulation (Philip and Vijayan, 2015). Furthermore, using an *in silico* approach, glucocorticoid response elements in the promoters of SOCS genes in rainbow trout (Philip and Vijayan, 2015) were identified. It was also proposed that cortisol may act through SOCS in hepatocytes to limit growth hormone signalling (Philip and Vijayan, 2015). In support of this, upregulation of SOCS2 transcripts by cortisol corresponded with inhibition of growth hormone-mediated IGF (insulin-like growth factor) expression in trout hepatocytes (Philip and Vijayan, 2015). Based on these observations, a working hypothesis is that cortisol regulation of SOCS may be a mechanism by which glucocorticoids programme hepatocytes to redistribute substrates away from growth and immune function, and towards pathways essential for stress coping (Philip and Vijayan, 2015).

8.4 Targeted Secondary Stress Response

In addition to the non-specific physiological and biochemical stress responses described earlier, animals have also adapted specialized mechanisms by which to deal with specific thermal and osmotic stressors, which occur with climate change. As these specific responses usually include regulation of transcriptional and translational processes (Basu *et al.*, 2002; Podrabsky and Somero, 2004; Kültz, 2015), they impart a huge metabolic load on the organism, which may impact the metabolic response to stress. For example, this may be particularly significant for stenothermal fish in higher latitudes, such as the Arctic or Antarctic, where environmental changes are generally less dramatic (Windisch *et al.*, 2011; Huth and Place, 2016).

8.4.1 The heat shock response

A key acute secondary response at the cellular level is the rapid synthesis of a suite of proteins that are instrumental in cellular protein homeostasis. The cellular stress response involves the upregulation of several classes of heat shock proteins (Hsps), generally categorized as the heat shock response (Richter *et al.*, 2010). Stressors, such as heat, have several deleterious effects at the cellular level such as reorganization or aggregation of cytoskeleton components. This leads to the loss of correct organelle localization, a decrease in transcription and translation as well as an increase

in membrane permeability causing changes in intracellular pH and ion concentrations (Richter *et al.*, 2010). The induction of Hsps are adaptive and they provide cytoprotection to offset the proteotoxicity in response to stressors by acting as molecular chaperones (Iwama *et al.*, 1998; Deane and Woo, 2011). The heat shock response is conserved among species; however, the composition and rate of upregulation of each class of proteins will vary (Richter *et al.*, 2010). The subset of the Hsp family, whose primary role is to act as molecular chaperones, are divided into five families based on molecular mass, including Hsp27, 60, 70, 90 and 110 (Kregel, 2002; Calderwood *et al.*, 2007; Richter *et al.*, 2010). These stress proteins comprise 5–10% of total protein in an unstressed cell but can be induced to 15% of total protein during stress (Calderwood *et al.*, 2007; Pockley *et al.*, 2008). The Hsps are primarily known for their intracellular roles in cell signalling and molecular chaperoning (Parsell and Lindquist, 1993). Hsp70 (68–73 kDa) has been extensively studied, and is the most highly conserved of the Hsps (Pockley and Multhoff, 2009). Within this family, there are two main isoforms: (i) Hsc70 which is constitutively expressed in all cells; and (ii) the inducible Hsp70 that is synthesized rapidly in response to denatured proteins. Hsc70 is involved in proper protein folding, intracellular localization and secretion (Feder and Hofmann, 1999), while the intracellular role of Hsp70 includes molecular chaperoning, folding nascent polypeptide chains, mediating the repair or degradation of denatured proteins, and protein transport (Kregel, 2002). Although Hsps were first observed in response to heat shock, we now know that any stressor affecting protein stability will stimulate the synthesis of these proteins in various fish species (Deane and Woo, 2011).

The regulation of the heat shock response and synthesis of Hsps occurs through the activation of heat shock factors (HSF), which will bind to the promoter region of heat shock genes during stress, initiating transcription. There are four possible heat shock factors; however, HSF1 is the principal transcription factor in mammals and lower vertebrates (Sarge *et al.*, 1993; Deane and Woo, 2011), while HSF4 is constitutively expressed (Nakai *et al.*, 1997) and HSF2 expression appears developmentally regulated (Pirkkala *et al.*, 2001). Within avian species, HSF3 is the main transcription factor induced by stress (Pirkkala *et al.*, 2001). In the absence of stress, HSF1 is found within the cytoplasm in a latent monomeric state bound to HSP70 (Kregel, 2002). Upon detection of a stressor, the

Hsp70 or ubiquitin is removed from HSF1 leading to homotrimerization and subsequent transport to the nucleus where it is hyperphosphorylated by several kinases (Richter *et al.*, 2010). The trimer can then bind to the heat shock element (HSE), located in the promoter region of the Hsp70 gene, inducing transcription. Regulation of HSF1 binding to the HSE is mediated by heat shock binding protein 1 which can be subject to negative feedback by Hsp70 thereby repressing synthesis (Shi *et al.*, 1998).

The role of Hsps in stress adaptation in fishes has received a lot of attention (see reviews by Iwama *et al.* (1998) and Deane and Woo (2011)). In the aquatic environment, fish are exposed to a variety of stressors, including diurnal or seasonal changes in temperature, limited dissolved oxygen and pollutants (Deane and Woo, 2011). Consequently, the capacity to synthesize Hsps may be instrumental in determining the stress level and health of an organism, and subsequently the quality of its environment (Deane and Woo, 2011). Hsps have been studied in a variety of fish, and they all show inducible Hsp70 in response to temperature changes, while the magnitude of response is dictated by the intensity and the duration of the stressors, as well as the tissue and species specificity (Deane and Woo, 2011). Overall, Hsp induction is energy demanding as protein synthesis is thought to account for more than 50% of the energy budget of a cell (Houlihan, 1991). Consequently, the induction of this protein is critical for cells to cope with stress, and conversely the lack of induction of Hsp70 is associated with compromised cellular performance and survival (Deane and Woo, 2011). In contrast to the inducible form, Hsc70 levels are relatively constant and expressed constitutively and unaltered by heat shock or chemical stressors (White *et al.*, 1994; Norris *et al.*, 1995; Currie *et al.*, 1999; Boone and Vijayan, 2002). However, an increase in Hsc70 mRNA was observed in zebrafish embryos and medaka (*Oryzias latipes*) cell lines in response to heat shock (Santacruz *et al.*, 1997), but whether this is translated to protein is uncertain.

Physical stressors do not affect the expression of liver Hsp70, despite the transient elevation in cortisol and glucose levels after stressor exposure (Vijayan *et al.*, 1997; Washburn *et al.*, 2002). However, cortisol has been shown to attenuate the Hsp response. In mammals GR inhibits HSF1 activity, by reducing the HSF occupancy in the promoter region of heat shock genes (Li and Sánchez, 2005).

Interestingly, GR modulates intracellular Hsp70 levels in macrophages and fibroblasts in sea bream, and this was correlated with increased cell survival (Deane and Woo, 2007). In rainbow trout hepatocytes, it was demonstrated that while cortisol will not affect the rate of protein degradation, it will reduce *de novo* synthesis of Hsp70 post-stressor (Boone and Vijayan, 2002). Comparatively the constitutive form, Hsc70, is upregulated by cortisol in rainbow trout liver (Vijayan *et al.*, 2003), which may lead to an increase of the stress threshold in animals. Both cortisol and epinephrine attenuated the heat shock-mediated elevation in extracellular Hsp70 levels in red blood cells at 24 h post-heat shock exposure (Henrickson, 2010).

While Hsp70 is primarily an intracellular chaperone, it is also released into the extracellular environment. As Hsp70 is not tagged or secreted through classical mechanisms, it was thought that its release involved exosomes. Exosomes are extracellular vesicles, approximately 30–100 nm in diameter and contain protein and nucleic acids, reflective of their cell of origin. Hsp70 release in exosomes was recently shown in rainbow trout (Faught *et al.*, 2017). The physiological role for Hsp70 release is unknown, but a recent study revealed that exosomal Hsp70 transmission across cell types may be a key mechanism for the maintenance of organismal proteostasis in *Drosophila melanogaster* (Takeuchi *et al.*, 2015). Indeed, in rainbow trout, stress and cortisol were able to modulate the release of Hsp70-positive exosomes (Faught *et al.*, 2017). The role of exosomal Hsps in the stress response is an interesting area of research and exosomal Hsps have the potential to be a primary stressor sensor that communicates with multiple tissues to mount an appropriate cellular stress response. For instance, Hsps and other proteins in the exosomes released from gills may prime other tissues, including liver and brain, to mount a protective response, including energy substrate repartitioning to cope with the stressor, but this remains to be established in fish.

8.4.2 Osmotic stress

Another energy demanding process associated with stress is the re-establishment or maintenance of osmotic and ionic balance in fishes, which is dependent on the ionic strength (salinity) of the medium (Morgan and Iwama, 1999; Ern *et al.*, 2014). Most fishes are constantly exposed to some

form of osmotic stress since they rarely live under isosmotic conditions and are thus faced with direct osmotic and ionic gradients across their body surfaces in contact with their surrounding aquatic environment (Takei and Hwang, 2016). Fish have adapted to living under either hyperosmotic (e.g. seawater) or hypoosmotic (e.g. freshwater) conditions (Schultz and McCormick, 2013). Consequently, with these gradients for water and ions between the fish and the environment, active compensatory mechanisms are in place to re-establish homeostasis and deal effectively with osmotic stress. These mechanisms are reviewed in Chapter 9 (this volume) and elsewhere (Marshall and Grosell, 2005; Evans, 2010; Takei and Hwang, 2016). Briefly, seawater fishes that are challenged with osmotic water loss (dehydration) and ion gain compensate by drinking seawater to rehydrate and excrete excess salts via gill chloride cells. The kidneys produce minimal amounts of urine to excrete excess divalent ions, but conserve water. In contrast, freshwater fishes faced with osmotic water gain and diffusive ion losses compensate by renal production of large volumes of dilute urine and branchial ion uptake by a number of specialized ionocytes (or ion transport cells). In fresh water, fish do not drink so the role of the gut in osmoregulation is limited to ion uptake during digestion (Wood and Bucking, 2010). Movement between hyperosmotic and hypoosmotic environments is particularly challenging and consequently less than 1% of species are capable of living under freshwater and marine conditions (Schultz and McCormick, 2013). The reorganization of the osmoregulatory organ functions requires remodelling of the respective epithelia with cell proliferation and death by apoptosis (Takei and McCormick, 2013). In fishes, osmosensing requires the integration of information from a number of osmosensors that perceive the osmotic stress so that the characteristics (magnitude, direction and ionic composition) of the osmolality change can be gauged and a suitable compensatory response enacted (Kültz, 2012). Potential osmosensors include the calcium sensing receptor (CaSR), transient receptor potential (TRP) cation channels and atypical voltage-gated Na⁺ channel Nax (Takei and Hwang, 2016).

The CaSR is a GPCR that binds divalent cations, such as Ca²⁺ and, since it is localized to body surfaces, it potentially functions as an osmosensor via divalent cation (Ca²⁺ and/or Mg²⁺) sensing (Nearing *et al.*, 2002; Loretz, 2008). In Mozambique tilapia,

salinity dependent changes in CaSR transcript levels in kidney and intestine have been observed (Loretz *et al.*, 2004). However, although CaSR is expressed in gill ionocytes in Mozambique tilapia (Loretz *et al.*, 2009) acclimatized to seawater, it did not alter CaSR transcript levels (Breves *et al.*, 2010). Consequently, it is not clear whether CaSR is an osmosensor. The TRP cation channels have been shown to sense Na⁺, Ca²⁺ and osmolality in fishes and other animals (Kültz, 2012). In particular the osmosensory TRP vanilloid 4 (TRPV4) is expressed in gill epithelial cells of euryhaline European sea bass in a salinity dependent manner suggesting an ability to sense a hypoosmotic stress (Bossus *et al.*, 2011). Nax has been proposed as a potential Na⁺ sensor based on mammalian studies although the orthologues have yet to be identified in any fishes (Takei and Hwang, 2016).

The salinity responsive element osmotic stress transcription factor (OSTF1, TSC22-3b) was first identified in the euryhaline Mozambique tilapia (Fiol and Kültz, 2007). OSTF1 has since been identified in a number of different species including black porgy (*Acanthopagrus schlegelii*), Nile tilapia (*Oreochromis niloticus*), medaka and Japanese eel (*Anguilla japonica*) (reviewed by Kültz, 2012). For example, Tse *et al.*, (2007) found that *A. japonica* gill pavement cells respond to hypertonic shock *in vitro* with an increase in *ostf* mRNA expression as well as Na⁺/K⁺-ATPase (NKA) α and β subunit, Na⁺:K⁺:2Cl⁻ cotransporter (NKCC) and Na⁺/H⁺ exchanger 1 (NHE1) protein expression. Tse *et al.* (2008) went on to find that basal expression levels were higher in gill ionocytes (chloride cells) than in pavement cells, and that transfer to seawater resulted in higher expression in both cell types.

All this tissue remodelling may play a role in increasing the energy demand for osmotic compensation. We can also view the relationship between stress and osmoregulation from the perspective of the effects of stress on osmo- and ion regulation. During acute stress, the release of catecholamines results in increased perfusion of the gills to meet increased oxygen demands but this has a secondary effect of increasing gill permeability to water and ions (McDonald *et al.*, 1991). This effect is referred to as the osmorepiratory compromise and is discussed in more detail in Chapter 9 (this volume), but in effect osmoregulation is compromised under these conditions (Gonzalez and McDonald, 1992; Gilmour and Perry, 2018). Exogenous cortisol and non-osmotic stressors (e.g. handling, confinement,

social interaction) that elevate plasma cortisol levels acting through the glucocorticoid receptor have profound effects on ion transporters and ionocyte populations. This is because the stress hormones have also been shown to directly affect ion transporter regulation (McCormick, 2001). In teleost fishes, cortisol is also the main mineralocorticoid and has been coined the seawater adapting hormone (McCormick, 2001) but it also plays a central role in regulating freshwater ion transport as well (Evans *et al.*, 2005; Takei and Hwang, 2016; Kwong *et al.*, 2016). The branchial chloride cell in seawater fish is responsible for the transcellular Cl⁻ secretion, proliferates and develops in response to cortisol, and NKA, the pump that drives this process, is also stimulated ((McCormick, 2001); see Fig. 9.2 in Chapter 9, this volume). Cortisol also increases the expression of the basolateral NKCC1, which is responsible for Cl⁻ uptake into the chloride cell (Pelis and McCormick, 2001) and the CFTR (cystic fibrosis transmembrane regulator) Cl⁻ channel that is involved in the excretion of Cl⁻ across the apical membrane (Marshall and Singer, 2002). In freshwater fish such as the zebrafish, cortisol stimulates ion uptake including the apical Na⁺/H⁺ exchanger 3 (NHE3), vacuolar-type H⁺-ATPase (VHA), Na⁺:Cl⁻ cotransporter (NCC) and epithelial Ca²⁺ channel (ECaC), in addition to the basolateral NKA (Kwong *et al.*, 2016; Takei and Hwang, 2016). Cortisol also stimulates proliferation of H⁺-ATPase-rich (HR), NCC-expressing and Na⁺/K⁺-ATPase rich (NR) ionocyte numbers (Kwong *et al.*, 2016). While from a stress standpoint cortisol may be a key regulator of ion homeostasis, we cannot discount the roles of other hormones, including growth hormone and prolactin (McCormick, 2001; Evans *et al.*, 2005), but their plasma changes and action during acute and chronic stress on ion regulation are not very clear.

Relatively few studies have been focussed on osmotic stress at the cellular level and the cellular stress response (Deane and Woo, 2011). The rationale being that an osmotic stress results in damage or disruption to the cellular proteins, leading to a compensatory Hsp response as mentioned earlier in Section 8.4.1 on the heat shock response. Using silver sea bream (*Sparus sarba*), Deane and Woo (2004) observed a positive correlation between branchial *hsc70*, *hsp70* and *hsf1* transcript expression and salinity (6–50 ppt). There was no correlation in kidney and only hepatic *hsc70* was significantly higher in hypersaline challenge versus isosmotic conditions (50 versus 12 ppt).

Since gills are directly exposed to environmental salinity, the cytoprotective effects of Hsp/Hsc70 would be warranted. Deane *et al.* (2002) had also found the lowest hepatic Hsp (60, 70 and 90) levels in black sea bream (*Mylio macrocephalus*) held under iso-osmotic conditions and elevated expression levels under hyposaline and hypersaline conditions. In black sea bream, the highest growth rates were also observed under iso-osmotic conditions (Kelly *et al.*, 1999), which with the Hsp data indicate the least stressful conditions were optimal for growth. Malakpour Kolbadinezhad *et al.* (2018) also showed that Hsp70 levels in the salt-secreting dendritic organ increase in Plotosidae catfish experiencing hypersaline conditions. Like gills, the dendritic organ in Plotosidae catfish is external and is exposed directly to the environmental salinity, and therefore may also be directly facing an osmotic stress. These compensatory cellular responses would contribute to the increased metabolic demand in response to these osmotic stressors.

8.4.3 Circadian rhythm as modulator of the stress response

The light cycle can impact the physiology of fish, especially growth and reproduction (Kråkenes *et al.*, 1991; Stefansson *et al.*, 1991; Appelbaum and Kamler, 2000; Almazan-Rueda *et al.*, 2005; Villamizar *et al.*, 2009, 2014; Di Rosa *et al.*, 2016). Different light regimes and wavelength may also modulate the stress response. For instance, studies carried out in goldfish (*Carrasius auratus*: Song *et al.*, 2016), Nile tilapia (Volpato and Barreto, 2001) and yellow perch (*Perca flavescens*: Head and Malison, 2000) showed that animals maintained under green light have less stress than those kept under red light. Indeed, both cyprinid and salmonid fishes can detect ultraviolet (UV) and polarization sensitivity, but older fish lose the ability to detect UV (Bowmaker and Kunz, 1987). In addition to light, temperature is also a key cue for physiological changes including reproduction. For instance, many species of cyprinids breed under conditions of extended light and increased water temperature. This integration of photoperiod and temperature and the associated physiological changes are the result of the circadian system (Refinetti, 1999). Organisms respond to environmental rhythmical changes in two ways: (i) the passive responses (on-off mode) that appear daily

and/or seasonal rhythmic fluctuations of an environmental variable (usually the photoperiod or temperature); and (ii) those rhythmic responses that persist even in the absence of cyclic external stimuli, under constant environment conditions. The latter rhythmical responses are driven by internal structures with autonomous functioning called biological clocks (Golombek and Cardinali, 1993). These internal clocks regulate a wide range of physiological activities, including the stress response (Roenneberg and Mellow, 2005).

The main machinery of the mammalian circadian clock is found in the suprachiasmatic nucleus (SCN) of the hypothalamus (Davidson *et al.*, 2008). In the case of fish, the circadian rhythm generating system consists of multiple coupled central oscillators, including the retina, pineal gland and hypothalamus (Menaker *et al.*, 1997; Falcón *et al.*, 2010; Lopez-Patino *et al.*, 2011). The basic mechanism involves a series of feedback loops between the 'clock genes' and their protein products (Panda *et al.*, 2002). According to the best-known model the circadian system starts with the accumulation of CLOCK and BMAL1 proteins in the cytoplasm (Panda *et al.*, 2002) which form a heterodimer (CLOCK/BMAL1) and binds to the E-box promoters in the target genes, including *per* and *cry*. This completes the negative branch of the loop. The higher expression of PER and CRY results in the formation of a heterodimer, which inhibits the transcription of the *clock/bmal1* complex (Kondratov, 2007). *Clock* genes have been identified in zebrafish (Cahill, 2002), goldfish (Velarde *et al.*, 2009), sea bream (Vera *et al.*, 2013), salmon (Betancor *et al.*, 2014) and rainbow trout (Lopez-Patino *et al.*, 2011; Hernández-Pérez *et al.*, 2017). In general, the molecular mechanisms appear to be highly conserved with a positive transcriptional loop (*clock* and *bmal1*) and a negative transcriptional loop (*per* and *cry*). However, an important difference between fish and other vertebrates is the existence of multiple copies of clock genes in teleosts due to the genome duplication in the actinopterygian lineage (Toloza-Villalobos *et al.*, 2015). The functional role for these orthologs remains to be determined.

Photoperiod is fundamental for the development of the circadian system (Vatine *et al.*, 2011), and both melatonin and cortisol appear to be the key mediator of the photoperiod response. Melatonin is one of the key molecules in the transmission of the rhythm signal by photoperiod (Vatine *et al.*, 2011),

which in turn is modulated by temperature (Iigo and Aida, 1995). Also, temperature modulates the melatonin responses in a circadian manner (Rensing and Ruoff, 2002; Falcón *et al.*, 2010; Vera *et al.*, 2013). Temperature effects on melatonin rhythms could be related to the daily rhythmic activity of the enzyme arylalkylamine N-acetyltransferase (AANAT), essential for rhythmic melatonin production (Falcón *et al.*, 2010). A recent study suggested an entrainment of the expression of *aanat2* by temperature in zebrafish (Lahiri *et al.*, 2014). However, in another species (*Esox lucis*), thermocycles were able to synchronize melatonin production rhythm under constant darkness (DD), but the rhythm disappears after removing the temperature cycles (Falcón *et al.*, 1994).

Indeed, temperature is one of the signals that could affect the circadian system. For instance, the daily rhythms of locomotor activity in zebrafish can be entrained by thermocycles alone under constant lighting conditions (López-Olmeda *et al.*, 2006). In addition, unlike what happens with other zeitgebers, temperature changes may directly affect the transcriptional and translational machinery of the clock genes, including protein phosphorylation (Liu *et al.*, 1997; Lahiri *et al.*, 2005). Focusing on the clock mechanism, temperature changes of 1°C or 2°C are capable of entraining biological rhythms. For instance, Lahiri *et al.* (2005, 2014) reported the ability of the thermo- (high temperature) and cryophases (low temperature) to entrain the rhythms of expression of several clock genes in both zebrafish larvae and cultured cells maintained under DD. Moreover, the profile observed in those larvae kept under thermocycles and constant darkness was very similar to the profile observed in larvae under an alternating light and dark cycle and constant temperature.

Little is known regarding the rhythmicity of the HPI axis in fish. There are studies showing rhythms in the circulating levels of ACTH in the goldfish (Singley and Chavin, 1976), and daily fluctuations of cortisol in several teleosts (Pickering and Pottinger, 1983; Cerdá-Reverter *et al.*, 1998; Pavlidis *et al.*, 1999; Ebbesson *et al.*, 2008; Montoya *et al.*, 2010). In general, cortisol secretion reaches a peak in the early morning, but is also synchronized to both the feeding-fasting cycle and feeding time in fish (Isorna *et al.*, 2017). Recent studies have shown the existence of cortisol rhythms in zebrafish larvae (Yeh, 2015). Also, GR signalling is thought to play an important role in

the glucocorticoid regulation of rhythm signals in peripheral tissues (Dickmeis *et al.*, 2007). In addition, cortisol also modulates the expression of clock genes, including upregulation of *per* and downregulation of *clock* and *bmal1* in the liver of goldfish (Sánchez-Bretaña *et al.*, 2017). Also, stress decreased the expression of clock genes in rainbow trout brain (Naderi *et al.*, 2018). Interestingly, this experiment showed that the action of cortisol through the GR could not be the only mechanism involved, since mifepristone was not able to reverse the changes caused by stress in the expression of clock genes (Naderi *et al.*, 2018). There is not much information in fishes about how clock genes affect cortisol and stress responses. In mice, a clock gene mutation in the positive limb of the oscillator, *bmal1* or *clock*, causes low levels of cortisol (Turek, 2005; Leliavski *et al.*, 2015), while mutations in the negative loop could reduce (*per2* mutations) or increase (*cry* mutations) cortisol levels (Yang *et al.*, 2009; Lamia *et al.*, 2011). All this leads to the proposal that circadian rhythm and the targets that control rhythmicity may play an important role in modulating the stress response. The integration of temperature in modulating the components of the circadian rhythm in fishes (Table 8.1) suggests that climate change, as it progresses, will continue to modulate the biological clock, and the associated physiological responses in fishes to seasonal and diel changes. Conceptually, we propose this as a reciprocal interaction between the circadian system and the HPI axis functioning that may determine whether the animal will be able to cope with the changing climate, and this may be dictated by various factors, including species, habitat, life history and stress sensitivity (Fig. 8.4).

8.5 The Quaternary Stress Response: Essential for Environmental Stress Adaptation?

The concept of allostasis, which broadly echos physiological stability by changes in the set-point of regulated variables, appears to be more in line with what is observed in animals adapting to stressors (McEwen, 2005; McEwen and Wingfield, 2010). The metabolic cost associated with the changes in the regulated variable, which may be essential for survival, is termed the allostatic load (McEwen and Wingfield, 2010). Allostatic state may involve altered and sustained activity levels of the primary endocrine response (i.e. corticosteroid), that is

Table 8.1. Effects of temperature and/or photoperiod on the stress response in fishes.

Species	Scientific name	Treatment	Stress effect ^a	Reference
Goldfish	<i>Carassius auratus</i>	Acute temperature changes	Increased energy demand in liver and muscle in the first 96 h post-stress	Gandar <i>et al.</i> (2017)
Red sea bream	<i>Pagrus major</i>	Chronic photoperiod manipulation	No change to plasma cortisol; growth enhanced	Biswas <i>et al.</i> (2006)
Nile tilapia	<i>Oreochromis niloticus</i>	Chronic photoperiod manipulation	No change to plasma cortisol; growth enhanced	Biswas <i>et al.</i> (2004)
Mozambique tilapia	<i>Oreochromis mossambicus</i>	Decrease temperature	Increased plasma cortisol levels	Fiess <i>et al.</i> (2007)
Arctic charr	<i>Salvelinus alpinus</i>	Decrease temperature	Repressed rhythms in liver transcriptome	Prokkola <i>et al.</i> (2018)
Senegalese sole	<i>Solea senegalensis</i>	Decrease temperature	Increased plasma cortisol levels	Costas <i>et al.</i> (2013)
Zebrafish	<i>Danio rerio</i>	Heat shock	Increased expression of clock genes	Jerônimo <i>et al.</i> (2017)
Common carp	<i>Cyprinus carpio</i>	Increase temperature	Increased plasma cortisol levels; increased POMC mRNA expression	Arends <i>et al.</i> (1998)
Brook charr	<i>Salvelinus fontinalis</i>	Increase temperature	Increased plasma cortisol levels	Chadwick and McCormick (2017)
Cod	<i>Gadus morhua</i>	Increase temperature	Migration to cold water; increased growth and fecundity	Pörtner <i>et al.</i> (2001)
Eelpout	<i>Zoarces viviparus</i>	Increase temperature	Migration to cold water; increase growth and fecundity	Pörtner <i>et al.</i> (2001)
Pacific sardine	<i>Sardinops caeruleus</i>	Increase temperature	Change in spawning	Sánchez-Velasco <i>et al.</i> (2002)
Goldfish	<i>C. auratus</i>	Increase in temperature (1 h)	Increased cortisol levels	Cockrem <i>et al.</i> (2019)
Cod	<i>G. morhua</i>	Temperature changes	No effect in plasma cortisol; similar stress response	Perez-Casanova <i>et al.</i> (2008)
Rainbow trout	<i>Oncorhynchus mykiss</i>	Total light along 2 months	Increased plasma cortisol	Leonardi and Klempau (2003)

^aPOMC, pro-opiomelanocortin.

essential for integrating the energy changes in response to environmental challenges (abiotic and anthropogenic). Hibernating bears accumulating fat prior to hibernation is an example of an allostatic load, which allows them to survive for specific periods of time if the stored energy is available (McEwen, 2005; McEwen and Wingfield, 2010). The question arises what happens when an animal in one of these allostatic states reproduces – will that confer an adaptive advantage to the progeny? There are studies in fish suggesting that this may be the case. The maternal transfer of transcripts and hormones from the perspective of stress and cortisol has been reviewed previously in fish (Nesan and

Vijayan, 2013; Faught and Vijayan, 2018b). The take-home message is that when the mother experiences stress, the maternal transfer of hormones, transcripts and metabolites changes and the resulting progeny may be affected, and these changes may be adaptive or maladaptive. Recent studies in coral reef fish (*Acanthochromis polyacanthus*) exposed to +3°C for one or two generations, saw a suite of differentially methylated regions based on the temperature at which the parents were held, suggesting epigenetic modification as a key mechanism for generational changes associated with climate changes (Bernal *et al.*, 2018; Ryu *et al.*, 2018). These workers showed that fish that are exposed to

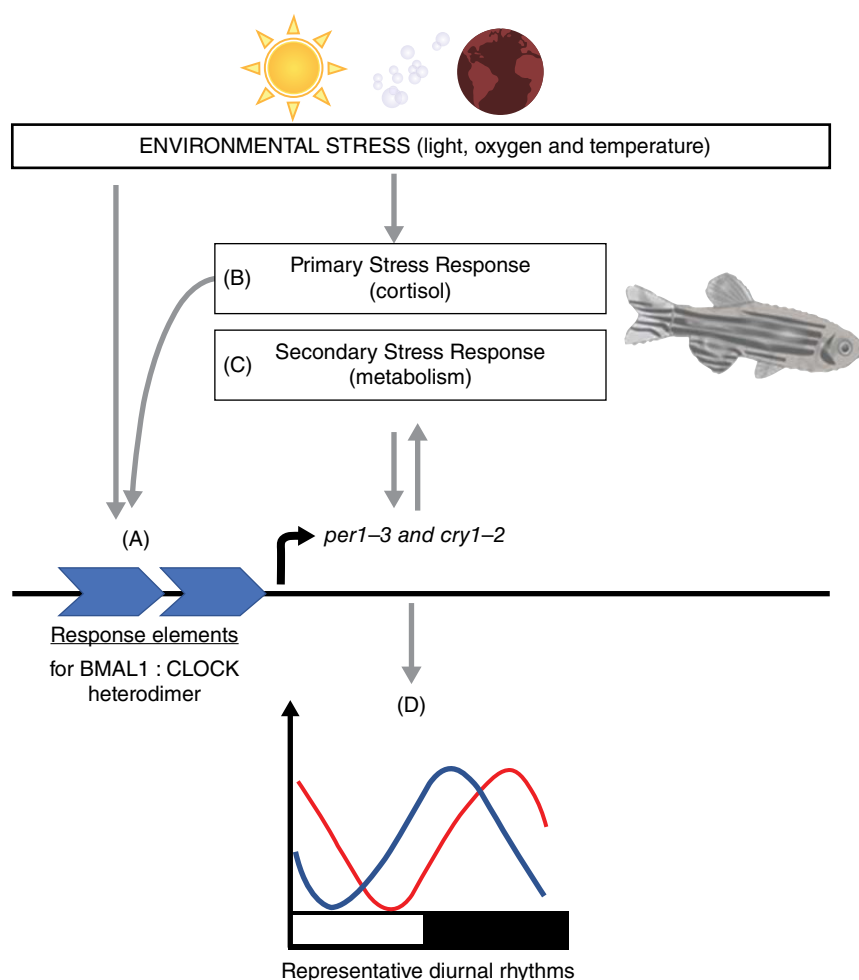


Fig. 8.4. Environmental changes influence circadian rhythms and the stress response in fish. (A) External cues, including photoperiod, oxygen and temperature, directly affect the circadian rhythm and stress response of fishes. BMAL1 and CLOCK transcription factors form heterodimers and bind to response elements (i.e. E-box elements (blue arrows)), to activate the transcription of *per* (1–3) and *cry* (1–2). PER and CLOCK will negatively inhibit their own transcription. (B) Ambient light can influence the diurnal secretion of cortisol, and cortisol in turn can modulate transcription of the *clock* genes, and their downstream targets. (C) Metabolism is also under circadian control as cellular energy sensors such as sirtuin1 and 5' adenosine monophosphate activated protein kinase (AMPK) can modulate the transcription of several *clock*, *per* and *cry* genes. (D) The graph represents the diurnal rhythm (blue line) of key hormones (leptin, ghrelin, cortisol) and metabolites (glucose, free fatty acids), which are under control of circadian molecular mechanisms. Circadian desynchronization (red line) due to environmental changes can be adaptive or maladaptive, and this may be dictated by the species resilience (i.e. heat tolerance), as well as their stress sensitivity. BMAL1, brain and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles kaput; *cry*, cryptochrome; *per*, period. (Adapted from Kumar Jha *et al.*, 2015.)

warmer water differentially express a similar suite of genes compared with fish that are exposed to elevated temperatures in just one generation (Ryu *et al.*, 2018). This suggests that changes may occur in the epigenetic machinery that may confer a dif-

ferent allostatic state on the progeny. Also, we now know that the stress response may be altered in subsequent generations based on parental or ancestral exposure to stressors (Vera-Chang *et al.*, 2018). While the original categories (primary, secondary

and tertiary) of the stress response were intended to describe the individual's response to a stressor, we propose that the parental contribution to the progeny stress response (quaternary stress response) may constitute an essential adaptation to cope with environmental stress. If the information conferred to the progeny is maladaptive this may result in reduced offspring survival or fitness. The degree to which an animal must change in response to stress has also been reviewed recently (Somero, 2010), and postulates that stenotherms, which have lost their regulatory mechanisms for coping with temperature changes, will be more sensitive to environmental shifts. In this case, there may be no quaternary stress response to environmental stress.

8.6 Conclusion and Future Directions

The impact of the environment on the stress response in fish is well established. From abiotic factors such as temperature and salinity to anthropogenic factors such as toxicants, the stress response to these stressors is necessary for the animal's survival. A global increase in temperature has the most potential to affect the stress response of animals. Fundamentally, the stress response is associated with an increased metabolic demand, and warming water may compound this effect by increasing the standard metabolic rate of the animal. In addition, warming temperature reduces oxygen solubility, and the associated decline in aerobic scope may limit the performance and reduce the fitness of the animals. However, it is unreasonable to generalize these effects because few studies have empirically tested a global warming scenario and its impact on the stress response of fishes. A recent study reported that European perch (*Perca fluviatilis*) held in +5–10°C warmer water, over three decades, had resting metabolic rates that were plastic and thermally able to compensate better than control fish. However, the upper critical heat limits, as measured by cardiorespiratory function, were less flexible (Sandblom *et al.*, 2016). It is important to note that this study was carried out with a temperate eurythermal fish, and given the differing species sensitivities to temperature fluctuations, we predict that stenothermal animals in the higher latitudes may be less resilient to temperature changes (Whiteley and Egginton, 1999; Whiteley *et al.*, 2006). Indeed, while the lack of an adrenergic and corticosteroid stress response in Antarctic fish may be beneficial to limit the resting metabolic demand in a cold

environment, corticosteroid and catecholamines are essential for energy substrate mobilization. Consequently, these fish may lack the necessary tool kit to metabolically adapt to warming temperatures. Indeed, theoretical models indicate that the potential impact of temperature may be more noticeable in the stenothermal species of the tropics and the high latitude fishes (Comte and Olden, 2017), and this may include loss of aerobic scope and the associated performance dysfunctions (Mark *et al.*, 2005). Therefore, studies testing the multi-stressor coping capacities of Antarctic fishes at warming temperatures may shed light on the adaptive capacities with respect to the energetics of the stress response. In addition to temperature, any changes in salinity, due to changes in global ice reserves, or decreased rainfall may have a large impact on the stenohaline (tolerate a narrow salinity range) teleosts. Dramatic changes in salinity may result in osmotic stress, and the associated metabolic demands on the animals. A reduction in freshwater inputs into estuaries, which can result in inversions (hyposaline to hypersaline), will challenge even the euryhaline fish that are found in these ecosystems (Kültz, 2012). As mentioned in this chapter apart from abiotic stressors, change in temperature may also impact the circadian rhythm of animals, which are tightly linked to the stress response and physiological performances (Fig. 8.4). However, we know very little about temperature impacts on the circadian systems and rhythmicity in animals. This is one area of study that needs further attention, and may be particularly relevant to Arctic animals, whose lifestyle is so tightly linked to temperature and photoperiod for growth and reproduction. While it is tempting to generalize the impact of environmental stressors on teleost metabolism, the wide ranges in their distribution and thermal preferences suggests that the stress hormones-driven metabolic processes may be species specific.

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9

Ionic Regulation

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9.1 Introduction

There is a consensus view that rapid changes in the Earth's climate are linked to rising atmospheric levels of carbon dioxide (CO₂), a heat-trapping greenhouse gas, driven by fossil fuel burning and deforestation. Since the start of the Industrial Revolution oceanic surface temperatures have risen by 0.3–0.6°C (Orr *et al.*, 2005) and with current end-of-the-century predictions sea surface warming will range from +1.7°C to +4.8°C (IPCC, 2014). Given that fishes are poikilothermic ectotherms, changes in water temperature will have direct effects on reaction rates and metabolic rate. There are clearly established effects of temperature on ion and acid–base regulation that will be discussed.

In addition, the oceans have also been important in buffering a third of atmospheric CO₂ added to the atmosphere, but this is far from benign and ocean pH has dropped by 0.1 pH units since the Industrial Revolution resulting in ocean acidification (OA) (Orr *et al.*, 2005). The Intergovernmental Panel on Climate Change (IPCC) (2014) is predicting seawater pH varying between –0.07 and –0.32 pH units over this century. By 2300, CO₂ levels will reach ~1900 µatm CO₂, and pH 7.75. There are also now more recent predictions that these changes may occur even more rapidly (McNeil and Sasse, 2016) and that coastal upwelling areas are already experiencing these conditions (Feely *et al.*, 2008). Although fishes are for the most part robust acid–base regulators, and the challenges presented by OA aquatic hypercapnia are well within the scope of their capacities to successfully regulate, there have been some surprising findings on deleterious effects to behaviour and otolith growth that will be discussed.

Changes in climate will also impact environmental salinity through increased evaporation and decreased rainfall in arid regions on the one hand and increased freshwater input through precipitation and ice melt on the other (Knowles and Cayan, 2002; Schmitt, 2008). With the melting of the polar ice caps, sea levels are also predicted to rise resulting in inundation of coastal areas and salinization of freshwater habitats (Hallett *et al.*, 2018). These changes in salinity have obvious effects on fish ion regulation. It is not that any of these impacts are new, only that their frequency and severity will increase, in particular in the coastal zone and inland habitats. There is a well-established literature to support our understanding of what impacts climate-associated salinity changes will have on fishes (reviews by Evans *et al.*, 2005; Marshall and Grosell, 2006; Hwang and Lin, 2014; Takei *et al.*, 2014). Although salinities are predicted to change in the open ocean (Schmitt, 2008; Durack *et al.*, 2012), the changes are generally too small to be of significance to fish ion regulation.

In this chapter we will explore how climate change may impact ionoregulation in fishes. Ionoregulation in fishes is essential for survival and success, and osmoregulatory failure is often observed as a precursor to death. The two main climate change factors that are considered in this chapter are: (i) temperature; and (ii) CO₂-induced acidification. We will first provide the reader with a review of the basic mechanisms of ion regulation in marine and freshwater fishes, acid–base regulation, and their links. We will then review the literature on the predicted impacts of higher temperatures and aquatic hypercapnia on ion regulation in fishes. In the final part of the chapter we will look at the side effects

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of acid–base regulation on otolith growth and GABA_A (γ -aminobutyric acid, type A) receptor-associated behavioural changes. The chapter will close with some future directions in climate-change-associated research into ion regulation in fishes. Although there is consensus that these changes are occurring; predicting long-term impacts on fish populations is still a work in progress. There are also a number of recent reviews that we can recommend to the reader (Heuer and Grosell, 2014; Nilsson and Lefevre, 2016; Tresguerres and Hamilton, 2017; Esbaugh, 2018).

9.2 Marine Osmoregulation

With the exception of the hagfishes, marine fishes are engaged in some means of ion and osmoregulation. The hagfishes are stenohaline ion and osmoconformers that do not regulate extracellular Na⁺ and Cl[−] levels, which remain close to seawater levels (Clifford *et al.*, 2016). However, divalent ions are out of equilibrium with seawater indicating some regulation. Marine elasmobranchs, on the other hand, are osmoconformers and ion regulators. They use urea as an osmolyte that they retain in their tissues and extracellular fluids; however, to counteract the denaturing effects of urea on proteins, trimethylamine oxide (TMAO) is retained (Yancey, 2015). The elasmobranch kidney and gills are designed to retain urea, which is energetically costly to produce (Wilkie, 2002). Elasmobranch plasma ion concentrations (Na⁺ and Cl[−]) are maintained well below seawater concentrations through the secretion of a NaCl-rich solution by the rectal gland (Wright and Wood, 2015). Similar urea retention and salt-secreting tissues are also found in the chondrichthyan sister group of the elasmobranchs, the Holocephali (Hyodo *et al.*, 2007). Elasmobranchs are slightly hyperosmotic and are able to gain water through osmosis across their body surfaces, and thus do not generally actively drink for water balance.

Teleosts, the bony fishes, are all osmo and ionoregulators reflecting their freshwater ancestry. Plasma osmolality is regulated at roughly one-third of seawater values (Holmes and Donaldson, 1969). Under these conditions, prevailing osmotic and ionic gradients confront these animals with constant passive osmotic water loss and ion loading. Marine bony fishes actively compensate by drinking seawater and excreting excess ions via their gills (Fig. 9.1A).

9.2.1 Gill

In marine teleosts and lampreys, the gills are the primary salt-secreting organ. Concentrated in the gill filament epithelium are a population of large, ovoid-cuboidal shaped ionocytes that are characterized by an abundance of mitochondria, a well-developed branching tubular system continuous with the basolateral membrane rich in Na⁺/K⁺-ATPase (*Atp1a/Atp1b*) and a recessed apical opening or apical crypt (Wilson and Laurent, 2002; Wilson, 2011). These cells are named ionocytes, chloride cells (CCs) and mitochondrion-rich cells. They share the apical crypt with peripheral, less-developed mitochondrion-rich cells (= accessory cells) in the case of teleosts or other mitochondrion-rich cells in lamprey. Significantly, the shared tight junctions between these cells have fewer strands and present a more permeable paracellular pathway for ion fluxes. The CCs represent the transcellular pathway for secondary active Cl[−] secretion (Silva *et al.*, 1977; Evans *et al.*, 2005) (Fig. 9.2). The basolateral, electrogenic Na⁺/K⁺-ATPase uses ATP hydrolysis to pump two potassium ions (2 K⁺) into the cell in exchange for three sodium ions (3 Na⁺) out, lowering intracellular Na⁺ levels and contributing to the negative membrane potential. Cl[−] enters the cell via an electroneutral, basolateral Na⁺: K⁺: 2 Cl[−] cotransport-1 (NKCC1; *slc12a2*) using the inward Na⁺ gradient. K⁺ leaks out of the cell down its electrochemical gradient via a basolateral inwardly rectifying K⁺ channel. Intracellular Cl[−] exits the cell apically down its electrochemical gradient via a cystic fibrosis transmembrane regulator (CFTR; *abcc7*) Cl[−] channel. Na⁺ on the other hand takes a paracellular pathway. The transepithelial potential (TEP) in marine fishes is typically +20–30 mV, which is in the range of sodium's Nernst equilibrium potential calculated using plasma and seawater concentrations. The buildup of Na⁺ in the intercellular space from Na⁺/K⁺-ATPase pumping shifts the Na⁺ electrochemical gradient to favour paracellular Na⁺ excretion. This paracellular pathway for Na⁺ can be regulated by changing the expression of tight junction proteins with claudins Cld10e and Cld10d being associated with the sodium flux pathway (Kolosov *et al.*, 2013; Tipismark *et al.*, 2016; Marshall *et al.*, 2018). This model of salt secretion is present not only in marine fish gill CCs and presumably in seawater lamprey, but also the chondrichthyan rectal gland and the salt gland of other vertebrates (e.g. sea snake and

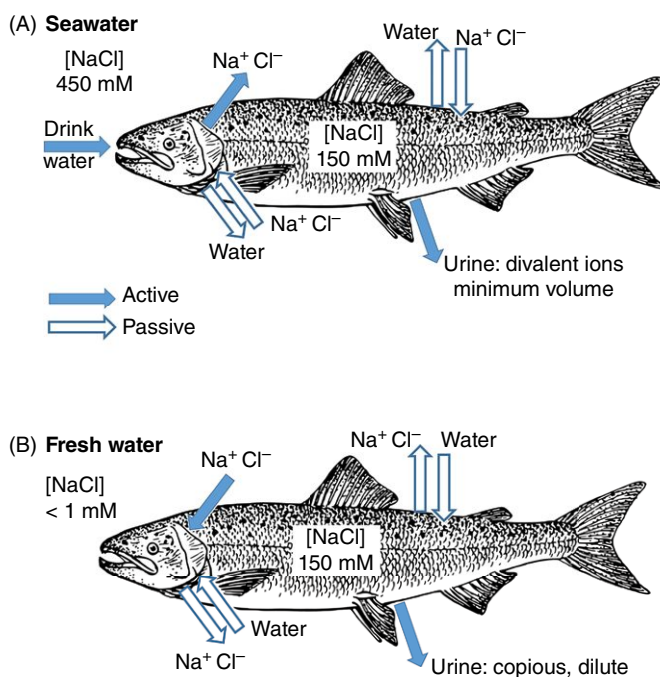


Fig. 9.1. Summary of the ion and osmoregulatory challenges and compensatory responses of (A) marine and (B) freshwater teleost fishes in addition to lampreys. Marine fishes face dehydration by osmosis, which they compensate for by drinking seawater. The passive salt uptake across their body surfaces and the additional salt load from drinking seawater is excreted across the gills by specialized ionocytes. The kidneys produce minimal amounts of urine to conserve water but excrete divalent ions. In contrast freshwater fishes are confronted with osmotic gain of water and loss of ions (Na^+ , Cl^-) across their body surfaces. They compensate by actively taking up ions across their gills and producing copious amounts of dilute urine to rid themselves of excess water.

salt water crocodile lingual glands, sea turtle lachrymal glands, sea bird and marine iguana nasal glands; Babonis and Brischoux, 2012).

In the elasmobranch fishes, the role of NaCl secretion for ion regulation is performed by the rectal gland (Wright and Wood, 2015). There is no evidence that the gills have a role in NaCl secretion. There are, however, mitochondrion-rich, intercalated cells in the gills but they have an amplified basolateral membrane through folding (basal labyrinth) rather than a tubular system as seen in teleost fishes (Wilson and Laurent, 2002). Two types have been defined based on the expression of ion transport proteins (Evans *et al.*, 2005): (i) the B-type or base-secreting cell expresses an apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger (Slc6a4; pendrin) and the basolateral V-type H^+ -ATPase (Piermarini and Evans, 2001; Piermarini *et al.*, 2002); and (ii) the A-type or acid-secreting cell expresses an apical Na^+/H^+ -exchanger-3 (NHE3; Slc9a3) and basolateral Na^+/K^+ -ATPase

(Choe *et al.*, 2005). These cells are involved in acid-base regulation and will be discussed further in Section 9.4.

9.2.2 Kidney

Osmoconforming hagfish do not show any indication of either water or NaCl reabsorption by their glomerular kidneys (Evans, 1979; Clifford *et al.*, 2016). However, the kidney of seawater lamprey has been shown to be able to produce hyperosmotic urine which has been associated with a countercurrent loop in the distal tubule region (McDonald, 2007). Marine elasmobranchs, which are typically slightly hyperosmotic to seawater and thus there is a positive water balance, have kidneys that typically absorb 70–85% of filtered water (Wright and Wood, 2015). The kidneys of elasmobranchs also have a complex organization into a countercurrent bundle that is designed to help

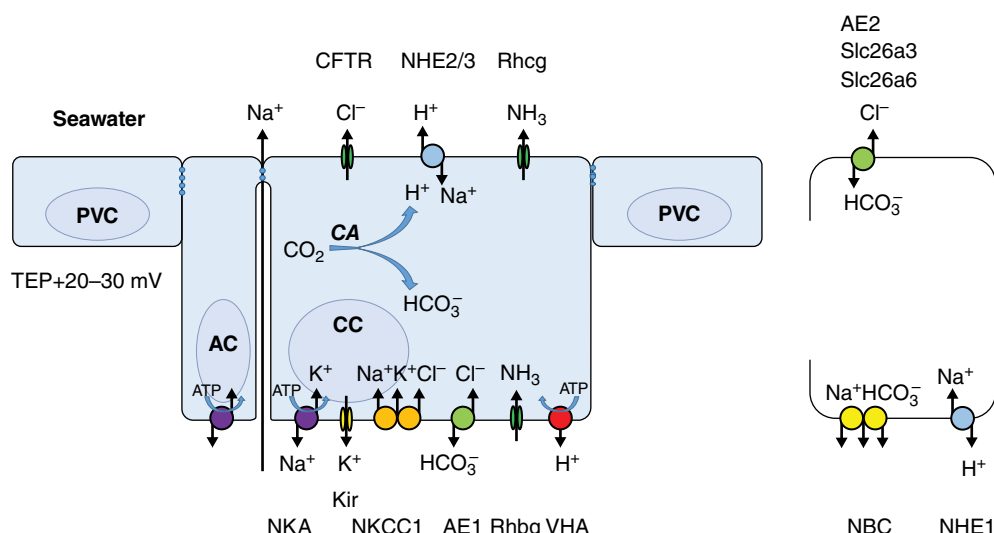


Fig. 9.2. Summary of the branchial chloride cell (CC) in marine teleost fishes. Secondary active Cl^- excretion is achieved by a basolateral Na^+/K^+ -ATPase (NKA), that creates favourable conditions for Cl^- uptake into the cells via a $\text{Na}^+:\text{K}^+:\text{Cl}^-$ -cotransporter (NKCC1). K^+ cycles through a basolateral Kir channel or exits apically through a ROMK (renal outer medullar potassium) K^+ channel. Intracellular Cl^- exits the cell across the apical membrane down its electrochemical gradient with a CFTR (cystic fibrosis transmembrane regulator) Cl^- channel. Na^+ on the other hand takes a paracellular route through the epithelium via permeable tight junctions between the CC and neighbouring accessory cells. In addition, CCs also express apical Na^+/H^+ exchanger (NHE2 and/or NHE3), Rh C glycoprotein (Rhcg) and basolateral Rh B glycoprotein (Rhbg). Basolateral V-type H^+ -ATPase, $\text{Na}^+:\text{HCO}_3^-$ cotransporter (NBC) and apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger (AE2, Slc26a6, Slc26a3) may also be present. AC, accessory cell; AE, anion exchanger; CA, carbonic anhydrase; Kir, inward rectifier potassium channel; PVC, pavement cell; TEP, transepithelial potential; VHA, vacuolar-type H^+ -ATPase. (Based on Evans *et al.*, 2005, Hiroi *et al.*, 2008 and Hwang and Lin, 2014.)

retain urea (Lacy and Reale, 1995). The kidneys are not a major site of NaCl secretion (Wright and Wood, 2015).

The kidney of marine teleost fishes lacks a concentrating mechanism such as the loop of Henle and therefore cannot produce hyperosmotic urine (relative to plasma) (McDonald, 2007). As a consequence, urine volumes are kept to a minimum by minimizing glomerular filtration rates either by changing the amount of blood flowing into the glomerulus or by changing the number of glomeruli being perfused in order to conserve water. Some marine fishes lack glomeruli altogether and all urine is formed through secretion (Beyenbach, 2004). The filtrate is modified in the proximal tubule (PT) with some NaCl and water reabsorption in PTI and secretion of Na^+ , Cl^- , Mg^{2+} and SO_4^{2-} in PTII (McDonald, 2007). In addition, the distal tubule, which is typically prominent in freshwater fishes for NaCl reabsorption, is absent in stenohaline marine teleost fishes (McDonald, 2007). However, the kidneys still play a critical role

in the excretion of toxic divalent ions in marine fishes (Ca^{2+} , Mg^{2+} and SO_4^{2-}).

9.2.3 Extrabranchial salt-secreting organs

Although the gills are the central NaCl -secreting organ in the vast majority of teleost fishes, the Plotosidae catfishes have a salt-secreting dendritic organ (Malakpour Kolbadinezhad *et al.*, 2018). The dendritic organ is a fleshy, external tuft of glandular tissue on the urogenital papillae that has mitochondrion-rich acinar parenchymal cells. In chondrichthyan fishes, the rectal gland functions as the main extra-branchial salt-secreting organ (Wright and Wood, 2015). In common with the gill CCs of marine teleosts, the parenchymal cells of the dendritic organ and rectal gland have high basolateral Na^+/K^+ -ATPase, and NKCC1 expression and an apical CFTR Cl^- channel. In contrast with typical marine teleost fishes, the gills of both chondrichthyan and Plotosidae catfishes have fewer ionocytes and they lack the machinery for NaCl secretion.

9.2.4 Drinking

Drinking in marine teleosts is stimulated by reductions in blood volume and elevations in plasma osmolality associated with dehydration (Carrick and Balment, 1983). Marine teleosts drink continuously at approximately 1–5 ml/kg/h (Whittamore, 2012; Grosell, 2014). The imbibed seawater is first desalinated by the oesophagus, which is highly permeable to Na^+ and Cl^- but not water. Towards the distal end of the oesophagus, active transport by the columnar epithelial cells in a process involving Na^+/H^+ -exchanger-2 (NHE2) contributes to NaCl absorption (Esbaugh and Grosell, 2014). The desalinated water that enters the intestine is absorbed via a solute-linked water transport mechanism whereby the transepithelial transport of ions draws water along into the lateral intercellular spaces where localized hypertonicity occurs (Larsen *et al.*, 2009). The basolateral Na^+/K^+ -ATPase creates favourable electrochemical conditions for apical $\text{Na}^+ : \text{K}^+ : 2\text{Cl}^-$ cotransporter-2 (NKCC2), $\text{Na}^+ : \text{Cl}^-$ cotransporter (NCC) and sodium-glucose linked transporter-1 (SGLT-1) to facilitate apical ion movements that result in the absorption of 39–85% of imbibed water (Whittamore, 2012). In contrast, divalent ions (Ca^{2+} , Mg^{2+} and SO_4^{2-}) are not actively absorbed so they will be concentrated in the intestinal lumen, presenting a potential problem for the osmotic uptake of water. To address this problem, luminal pH is increased (up to pH 9) through bicarbonate secretion via an apical $\text{Cl}^-/\text{HCO}_3^-$ exchange which results in the precipitation of 20–60% of the total excreted divalent cations as CaCO_3 and MgCO_3 (Wilson *et al.*, 2002; Whittamore *et al.*, 2010). The *slc26a6* has been identified as the apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger (Grosell, 2006). Apical H^+ secretion via an H^+ -ATPase has been shown to enhance water uptake by titrating some of the excreted HCO_3^- to allow for continued Cl^- uptake via $\text{Cl}^-/\text{HCO}_3^-$ exchange and thus solute-linked water uptake (Grosell, 2014).

In non-teleost fishes in seawater, drinking has been measured in euryhaline lamprey (Rankin, 1997) but not in hagfish although the latter have a high water permeability across their body surfaces (Glover *et al.*, 2017). Since elasmobranch fishes are iso-osmotic to slightly hyperosmotic to seawater under routine conditions, they are not under threat of dehydration, so drinking is unnecessary; however, in response to increased environmental salinity, or blood volume loss elasmobranch fishes have been shown to increase drinking rate (Anderson *et al.*, 2002).

9.3 Freshwater Osmoregulation in Fishes

In fresh water, all fishes are active osmoregulators but are confronted with the opposite osmoregulatory challenges to marine fishes (Fig. 9.1B). The inward osmotic gradient across a fish's body surfaces results in a net water gain, and the outward diffusive gradients for ions result in their passive losses. In general, the gills of freshwater fishes are less permeable compared with seawater fishes, but these passive fluxes none the less require compensatory mechanisms to maintain homeostasis. The gills are the primary site for the compensatory uptake of ions, and the kidneys are involved in the formation of copious amounts of dilute urine (Marshall and Grosell, 2006; Takei *et al.*, 2014). In non-feeding fishes, the intestine has a minor role in osmoregulation since fish do not drink in fresh water (Grosell, 2014; Takei *et al.*, 2014).

9.3.1 Gill

There have been significant advances in our understanding of the transport mechanisms involved in freshwater fish ion regulation in the past decades brought about in no small part by advances in genomics. In freshwater fishes, there are multiple mechanisms at play to facilitate ion uptake, which contrasts with the highly conserved secondarily active chloride secretion mechanism in seawater fishes (Section 9.2.1) (Silva *et al.*, 1977). A number of in-depth review articles have been written over the past decade outlining the increasing complexity of the ion transport protein repertoires of the gill ionocytes (Evans *et al.*, 2005; Dymowska *et al.*, 2012; Hiroi and McCormick, 2012; Hwang and Lin, 2014). Early on, these cells were recognized as potentially important sites for ion regulation with their abundant mitochondria for the supply of ATP for ion-motive ATPase or pumps, and the increased basolateral membrane surface area through either branching, anastomosing tubules (tubular system) or folding (basal labyrinth) (Wilson and Laurent, 2002). Typically, the amplified basolateral membrane is associated with high Na^+/K^+ -ATPase activities in these cells and in the gills in general (McCormick, 1995). Morphologically, these cells are similar to those found in seawater teleosts.

The subtypes of teleost gill ionocytes are summarized in Fig. 9.3. Sodium and chloride are dominant ions and fluxes are linked either directly or indirectly

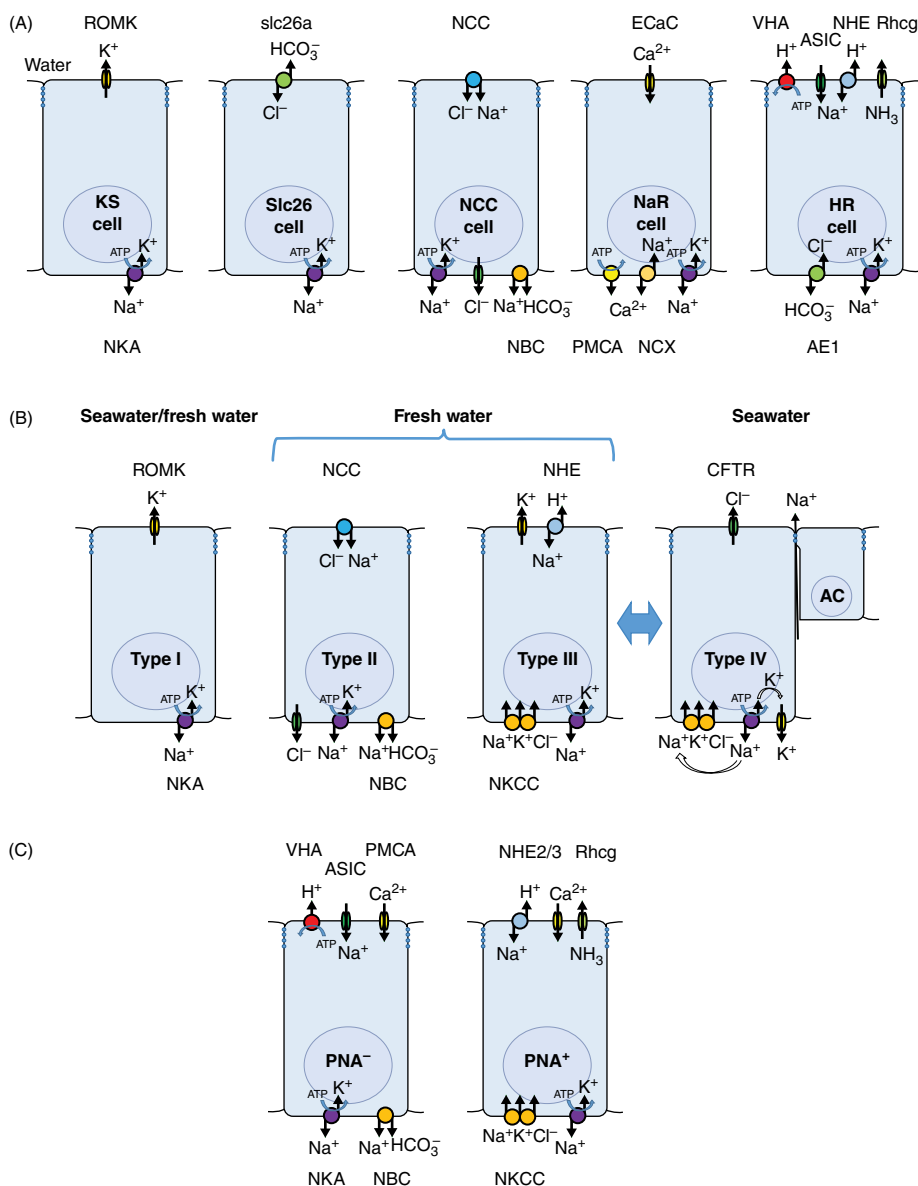


Fig. 9.3. Summary of branchial ionocyte types identified in teleost fishes. (A) The model stenohaline freshwater zebrafish (*Danio rerio*): the KS (potassium secreting) cell excretes excess K⁺ via ROMK K⁺ channel; the Slc26 cell absorbs Cl⁻ via a Cl⁻/HCO₃⁻ exchanger; the Na⁺: Cl⁻ cotransporter (NCC) cell; the Na⁺/K⁺-ATPase-rich (NaR) cell with the molecular machinery for Ca²⁺ uptake (apical epithelial Ca²⁺ channel (ECaC), and basolateral Na⁺/Ca²⁺ exchanger (NCX) and plasma membrane Ca²⁺-ATPase (PMCA)); and H⁺-ATPase-rich (HR) cell involved in Na⁺ uptake via direct coupling of Na⁺/H⁺ exchange via NHE or indirectly via the H⁺-ATPase and acid-sensing ion channel (ASIC) Na⁺ channel. (B) The euryhaline Mozambique tilapia (*Oreochromis mossambicus*) Type I, Type II, Type III and Type IV (seawater type) ionocytes. (C) The euryhaline rainbow trout (*Oncorhynchus mykiss*) PNA (peanut agglutinin) lectin positive and negative ionocytes. See text for more details on individual transporters. AC, accessory cell; AE1, anion exchanger 1; CFTR, cystic fibrosis transmembrane regulator; NBC, Na⁺: HCO₃⁻ cotransporter; NKA, Na⁺/K⁺-ATPase; NKCC, Na⁺: K⁺: 2 Cl⁻ cotransport; NHE, Na⁺/H⁺ exchanger Rhcg, Rh C glycoprotein; VHA, vacuolar-type H⁺-ATPase. (Based on Evans *et al.*, 2005, Hiroi *et al.*, 2008 and Hwang and Lin, 2014.)

to the transport of acid-base equivalents hydrogen ions, or protons, (H^+) or bicarbonate (HCO_3^-). The Na^+/H^+ exchange mechanism can be tracked back to the seminal work by Homer Smith (1930) and August Krogh (1939) on ion regulation in fishes. It has since been shown that this mechanism may work by direct coupling of exchange via a Na^+/H^+ exchanger (NHE) belonging to the *slc9a* gene family, which includes nine members but it is the apically localized NHE2 (*slc9a2*) and NHE3 (*slc9a3*) that have been linked to acid secretion in teleost fishes (Perry and Gilmour, 2006; Hwang and Lin, 2014) and can be inhibited by amiloride and its analogues (e.g. EIPA; (5-(N-ethyl-N-isopropyl) amiloride); Kleyman and Cragoe, 1988). However, the process requires either a H^+ or Na^+ gradient to drive the exchange which presents some thermodynamic constraints on direct coupling especially under acidic freshwater (ion poor) conditions (Perry and Gilmour, 2006; Parks *et al.*, 2008). More recently, the coupling of the NHE-Rh glycoprotein into a transport metabolon has been proposed by Wright and Wood (2009) to explain functioning of the NHE under unfavourable conditions such as ion-poor, acidic water (low pH and low $[Na^+]$). The Rh C glycoprotein (Rhcg) functions as a NH_3 gas channel so that as NH_4^+ is deprotonated as NH_3 enters the channel, the local concentration of H^+ increases to create a favourable H^+ gradient to drive Na^+ uptake. There is also some evidence that the NHEs may function as NH_4^+/Na^+ exchangers, whereby the NH_4^+ drives exchange directly (Wilson *et al.*, 2013; Ito *et al.*, 2014).

Indirect coupling of Na^+/H^+ exchange has been proposed based on the frog skin model of sodium uptake (Avella and Bornancin, 1989; Lin and Randall, 1995). The apical H^+ -ATPase has been identified as a vacuolar type (V-type), which hyperpolarizes the apical membrane to create a favourable electrochemical gradient for Na^+ to enter the cell via a sodium channel against its chemical gradient. The identity of the sodium channel had remained elusive (Hiroi and McCormick, 2012) until the acid-sensing ion channel (ASIC) was identified in the rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) (Dymowska *et al.*, 2014, 2015). The ASIC inhibitor DAPI (4',6-diamidino-2-phenylindole) and diminazene were shown to inhibit Na^+ uptake, and *Asic4* was localized to trout peanut agglutinin lectin negative (PNA-) ionocytes and zebrafish H^+ -ATPase rich (HR) ionocytes.

Although initially discounted as a transport mechanism based on the uncoupling of Na^+ and

Cl^- uptake, a Na^+ : Cl^- cotransporter (*slc12a10* NCC) has been identified in the gills of some freshwater fishes (Hiroi and McCormick, 2012). In zebrafish, NCC is expressed apically in NCC-type ionocytes and low environmental Cl^- concentrations increase expression and Cl^- uptake rates, while the NCC inhibitor metolazone reduced Cl^- uptake (Wang *et al.*, 2009). Low Na^+ decreased expression. Notably, salmonids appear to lack this branchial transport mechanism. The thermodynamics of this transport mechanism have also been questioned (Parks *et al.*, 2008).

The sodium uptake mechanism (NHE, H^+ -ATPase/ASIC or NCC) employed depends on the environmental Na^+ concentration with the H^+ -ATPase/ASIC operating under low Na concentrations. However, pharmacological and *asic4.2b* knock-down approaches in larval zebrafish did not reduce Na^+ uptake suggesting no role in Na^+ uptake; however, compensatory increases in the other Na^+ uptake mechanisms were observed (NHE3b and/or NCC) although not the numbers of NCC cells (Zimmer *et al.*, 2018). In *Fundulus heteroclitus* (mummichog or Atlantic killifish), which lacks an apical H^+ -ATPase, Brix *et al.* (2018) have suggested that the NHE2 was sensitive to DAPI.

The intracellular pool of H^+ is also produced by the hydration of CO_2 catalysed by cytosolic carbonic anhydrase (Gilmour, 2012). The carbonic anhydrase inhibitor acetazolamide has been shown to decrease acid excretion (e.g. Liu *et al.*, 2016). During acid excretion the H^+ exits the cell apically, while the HCO_3^- exits basolaterally via an electrogenic Na^+ : HCO_3^- cotransporter (NBC; *Slc4a4*) or Cl^-/HCO_3^- anion exchanger 1 (AE1; *Slc4a1*) (Liu *et al.*, 2016). In this way a net efflux of acid results. In contrast, in HCO_3^- or base-secreting cells of the gill, an apical Cl^-/HCO_3^- anion exchanger is present that belongs to either the *slc26a3*, *slc26a4* and/or *slc26a6* gene families based on gene knock down studies in zebrafish (Bayaa *et al.*, 2009). In these studies, the *Slc26a3* mediated Cl^- uptake under normal water Cl^- conditions. A more recent study in rainbow trout confirms the *Slc26a6* as a Cl^- uptake mechanism important in ion regulation (Boyle *et al.*, 2015). So far direct immunohistochemical localization studies have yet to show an apical localization of Cl^-/HCO_3^- anion exchangers in any teleost fish. In contrast, in the gills of elasmobranch fishes, the apical anion exchanger has been identified as the *Slc26a4* (pendrin) and localized to mitochondrion-rich intercalated cells that

also have high expression of basolateral V-type H⁺-ATPase (Piermarini *et al.*, 2002; Roa *et al.*, 2014). In response to a metabolic alkalosis (base infusion) in dogfish, translocation of H⁺-ATPase from intracellular vesicles to the basolateral membrane has been observed which correlates with increased base secretion (Tresguerres *et al.*, 2005).

The gill A and B type intercalated mitochondrion-rich cells described in marine elasmobranchs (Section 9.2.1) are also found in their freshwater counterparts (Ballantyne and Robinson, 2010). Expression of these ion transporters are also higher in euryhaline species in fresh water indicating a role in Na⁺ and Cl⁻ uptake (Piermarini *et al.*, 2002; Choe *et al.*, 2005; Evans *et al.*, 2005).

In freshwater lamprey, the cellular structure of gill ionocytes differs from that of teleosts although functionally they are remarkably similar (Bartels and Potter, 2004). Instead of having a tubular system, freshwater ionocytes have basolateral membrane infoldings (Bartels and Potter, 2004). These cells are referred to as intercalated cells and have been shown to express apical H⁺-ATPase, cytosolic carbonic anhydrase, and/or basolateral Na⁺/K⁺-ATPase (Choe *et al.*, 2004; Reis-Santos *et al.*, 2008; Ferreira-Martins *et al.*, 2016).

Calcium

Calcium is essential for cell metabolism, playing key regulatory roles, and is also important for bone growth in fishes (Flik *et al.*, 1995; Guerreiro and Fuentes, 2007). Consequently, both intracellular and extracellular levels are tightly regulated. In both freshwater and seawater fishes, the gills are the central site of active uptake. In freshwater fish, 97% of calcium uptake is branchial and even though seawater concentrations (10 mM) of calcium are higher than levels in the fish (2–4 mM), the electrochemical gradient results in passive losses of calcium that require an active compensatory uptake. Dietary uptake of calcium is not essential unless water calcium levels are very low (NRC, 2011). The mechanism of calcium uptake is well characterized and takes place in specialized branchial ionocytes (Na⁺/K⁺-ATPase-rich (NaR) cell type, Fig. 9.3; Hwang and Lin, 2014). Calcium uptake involves an apical epithelial calcium channel (ECaC; *trpv5*) that facilitates calcium uptake from the water, and a basolateral plasma membrane Ca²⁺-ATPase (PMCA; *atp2b*) and Na⁺/Ca²⁺ exchanger (NCX; *slc8a*) (Hwang and Lin, 2014).

The Na⁺/K⁺-ATPase in NaR cell-type ionocytes is important for creating the favourable gradients for the NCX to function. In zebrafish acclimatized to calcium-poor water, ECaC and Na⁺/K⁺-ATPase have been shown to increase in expression (Liao *et al.*, 2007). The same transport mechanism has been described in the intestine and gills of seawater fishes (Flik *et al.*, 1995; Guerreiro and Fuentes, 2007). However, in the case of the intestine of seawater teleost fishes, precipitation of imbibed calcium is important for water uptake (Whittamore, 2012; Grosell, 2014). The calcium sensing receptor (CaSR) has been shown to be important in regulating calcium transport (Hwang and Lin, 2014).

Potassium

Potassium homeostasis is essential for maintaining potential gradients across cells, especially in excitable tissues. The K⁺ uptake rates in freshwater fishes are only about 5% of Na⁺ and Cl⁻ (Eddy, 1985; Gardaire *et al.*, 1991; Gardaire and Isaia, 1992), and in feeding fishes diet would be the primary source of K⁺ for homeostasis (Buckling and Wood, 2006). An ionocyte subtype has been identified to be the site of K⁺ efflux in medaka (*Oryzias latipes*) (Horng *et al.*, 2017), zebrafish (Abbas *et al.*, 2011) and tilapia (*Oreochromis mossambicus*) (Furukawa *et al.*, 2014) expressing the renal outer medullar potassium channel (ROMK_a, also called *kcj1* or *kir1.1*). More recently Horng *et al.* (2017) have found evidence for an apparent paracellular K⁺ uptake pathway in medaka using the scanning ion electrode technique (SIET) and the paracellular blocker TAP (2,4,6-triaminopyrimidine), however, the TEP would need to be greater than -59 mV based on calculations of the K⁺ Nernst potential (Potts, 1984; average TEP of freshwater fishes -18 mV; range -54 mV to +5.5 mV). In Nile tilapia (*Oreochromis niloticus*), we have found that the gastric H⁺/K⁺-ATPase is expressed apically in a subpopulation of gill ionocytes and that in *ex vivo* gill preparations, Rb⁺ (a surrogate flux marker for K⁺) uptake is inhibited by the gastric proton pump inhibitors SHC28080 and omeprazole suggesting an alternative transcellular mechanism (Barnawi and Wilson, 2018).

9.3.2 Kidney

The kidneys of freshwater fishes produce copious amounts of dilute urine to rid the body of osmotically

absorbed water. This is done through high glomerular filtration rates (4 ml/kg/h; McDonald, 2007). The majority of NaCl in the filtrate is reabsorbed by the distal tubule (90%) by apical Na⁺: K⁺: 2 Cl⁻ cotransport (NKCC2) driven by basolateral Na⁺/K⁺-ATPase (Dantzler, 2003).

9.3.3 Intestine

Typically, ion flux experiments have been performed on fasted animals, and since freshwater fish do not drink, the role of the intestine under these conditions has been considered insignificant. However, more recently attention has shifted to the importance of dietary sources of ions in ion balance in freshwater fishes (review by Wood and Bucking, 2010). In fed fish, Bucking and Wood (2006) found in their studies on rainbow trout that over 80% of dietary Cl⁻ and K⁺ were absorbed, however, Na⁺ was not. The dietary source of Cl⁻ may be of particular importance to species that lack appreciable branchial Cl⁻ uptake such as the eel and killifish (e.g. *Anguilla rostrata*, Perry *et al.*, 1992; *F. heteroclitus*, Patrick and Wood, 1999).

There is also the unusual case of the male stickleback (*Gasterosteus aculeatus*), that modify their kidney tubules to produce mucus for nest building during the breeding season, and consequently shift fluid secretion to the gut (de Ruiter *et al.*, 1985).

9.4 Acid–Base Regulation in Fishes

Acid–base regulation is a key homeostatic process in fishes that has been reviewed extensively (e.g. Heisler, 1984, 1993; Claiborne, 1998, Claiborne *et al.*, 2002; Brauner and Baker, 2009). The normal range of blood and intracellular pH in fishes ranges between 7.7–8.0 and 7.2–7.5, respectively, and small changes can indicate a major acid–base imbalance (Claiborne, 1998). We will give a short review here to provide context for climate-change-associated impacts on ion regulation. In general, fishes cope with acid–base disturbances through buffering and by excretion/absorption of the acid–base equivalents H⁺ and HCO₃⁻ across their gills and to a lesser degree via their kidneys (Claiborne, 1998; Evans *et al.*, 2005). It is the latter coupling of ion and acid–base regulation that is of relevance for this chapter. The transport mechanisms involved were discussed previously in Sections 9.2.1 and 9.3.1.

9.4.1 Buffering

Buffering is important for dealing with the immediate impact of acid–base imbalances. Intracellular buffering capacity is determined primarily by fixed concentrations of inorganic and organic phosphates, amino acid residues and proteins that account for approximately 90% of the whole animal buffering capacity that are comparable to mammals (= non-bicarbonate buffers (NBB); Heisler, 1984; Claiborne, 1998). However, the HCO₃⁻ buffer system plays only a negligible role in intracellular buffering. In contrast, in extracellular fluids and blood, the HCO₃⁻ buffer system dominates and is enhanced by the enzyme carbonic anhydrase that reversibly catalyses the CO₂ hydration/dehydration reactions (CO₂ + H₂O ↔ H₂CO₃ ↔ HCO₃⁻ + H⁺). At physiological pH, the H₂CO₃ (carbonic acid) step is rapid. The CO₂ produced from metabolism in tissues is hydrated to HCO₃⁻ + H⁺ resulting in a drop in pH. The Henderson–Hasselbalch equation (Equation 9.1) is used to express the HCO₃⁻ buffer system:

$$\text{pCO}_2 = \text{p}K' + \log \frac{[\text{HCO}_3^-]}{\alpha_{\text{CO}_2} \text{pCO}_2} \quad (9.1)$$

where pCO₂ is the CO₂ partial pressure, pK' is the dissociation constant and α_{CO₂} is the CO₂ solubility coefficient. Values for constants can be calculated from equations derived by Boutilier *et al.* (1984) and Heisler (1984) and are notably temperature dependent. The Davenport or pH–HCO₃⁻ diagram (Fig. 9.4) is used to illustrate the relationship between blood pH, pCO₂ and [HCO₃⁻] during various acid–base disturbances (respiratory acidosis (A–B) and alkalosis (A–D), metabolic acidosis (A–C) and alkalosis (A–E)). A series of pCO₂ isopleths within the physiological range are calculated from the Henderson–Hasselbalch equation and experimental data is then plotted. Theoretical examples of different acid–base disturbances are presented. The fish with an approximate resting pH of 7.75, pCO₂ of 2 mm Hg¹ and [HCO₃⁻] of 5 mM is shown in Fig 9.4 (A). In practice either plasma pH and pCO₂ are measured and [HCO₃⁻] calculated, or plasma pH and total CO₂ content (C_{CO₂}) and pCO₂ calculated. In the latter case, pCO₂ and [HCO₃⁻] are calculated from C_{CO₂} using a rearrangement of the Henderson–Hasselbalch equation (Equations 9.2 and 9.3, respectively).

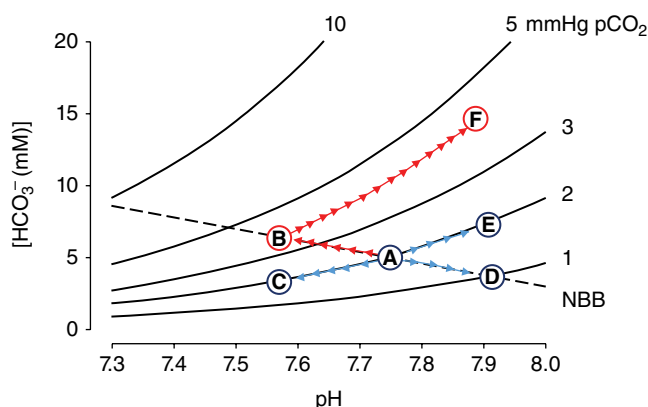


Fig. 9.4. Davenport pH- HCO_3^- diagram showing theoretical responses of a typical teleost fish to various acid-base disturbances. A $\rightarrow$ B: respiratory acidosis; A $\rightarrow$ C: metabolic acidosis; A $\rightarrow$ D: respiratory alkalosis; and A $\rightarrow$ E: metabolic alkalosis. The line B $\rightarrow$ F indicates the compensatory response to the respiratory acidosis. The non-bicarbonate buffer (NBB) line (-8 mmol HCO_3^- per pH unit). (Modified from Claiborne, 1998.)

$$\text{pCO}_2 = \frac{C_{\text{CO}_2}}{(10^{\text{pH}-\text{pK}'} + 1)\alpha\text{CO}_2} \quad (9.2)$$

$$[\text{HCO}_3^-] = C_{\text{CO}_2} - (\text{pCO}_2\alpha\text{CO}_2) \quad (9.3)$$

There are pros and cons for each approach, although the latter is now more frequently used (Heisler, 1984).

In blood, the non-bicarbonate buffers (NBB) are primarily erythrocyte haemoglobin and plasma proteins. Unlike in the NBB system, which is limited by the amount of buffers present, the HCO_3^- buffer system is open because CO_2 can be excreted by the gills as a normal part of respiration. However, in fish this system is complicated since gill ventilation is driven by the need to extract oxygen from the water due to the low solubility of oxygen in the water. In contrast CO_2 has a higher solubility in water and is readily lost to the ventilator water flow resulting in very low blood pCO_2 and consequently $[\text{HCO}_3^-]$. The combination of lower plasma $[\text{HCO}_3^-]$ and haematocrit (therefore haemoglobin concentrations) means that fishes' blood has a lower buffer capacity than their mammalian counterparts. This limits fishes in their ability to buffer fixed acid loads (metabolic acidosis). Also, since blood pCO_2 is already very low, there is limited although not insignificant capacity for a compensatory ventilatory response to eliminate CO_2 , which is primarily a mammalian strategy for compensating for a respiratory acidosis (Fig. 9.4 line

B-A) (Gilmour, 2001). Finally, relatively small increases in pCO_2 such as during hypercapnia will have more profound effects on blood pH compared with mammals (Claiborne, 1998; Fig. 9.4 line A-B). The new $[\text{HCO}_3^-]$ follows the slope of the non-bicarbonate buffering value (-8 mmol HCO_3^- per pH unit in the example in Fig. 9.4). To compensate for the blood acidosis associated with hypercapnia, fishes increase plasma $[\text{HCO}_3^-]$ (Fig. 9.4 line B-F) by acid (H^+) secretion or by increasing HCO_3^- uptake across the gills. These two processes are functionally equivalent and blood pH is typically returned to within 0.1–0.2 pH units within a matter of days. With graded hypercapnia, the increase in $[\text{HCO}_3^-]$ can be demonstrated up to a limit (Heisler, 1993).

9.4.2 Linkage between ion and acid-base regulation

Since respiratory compensation is limited, compensation to continuing acid-base perturbations relies primarily on acid-base equivalent transfers at the gills and kidney, with the gills dominating (90%). The kidney's contribution is limited by urine flow rates which are low in comparison with ventilatory gill water flow rates. This allows diffusion gradients across the gills to be maintained more easily. The mechanisms of H^+ and HCO_3^- transfers across the gill have been linked to electroneutral Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchange processes, respectively (Evans *et al.*, 2005; Hwang and Lin, 2014). In this way, ion and acid-base regulation are related and are interconnected.

9.4.3 Responses to aquatic hypercapnia

Historically aquatic hypercapnia or hypercarbia has been used to study acid–base regulatory mechanisms in fishes producing a wealth of data, but this has generally been done using more extreme and less environmentally relevant levels of CO₂ (Claiborne, 1998; Ishimatsu *et al.*, 2005). Typically, water is equilibrated with 1% CO₂ (~10,000 µatm = 7.6 mm Hg = 1.01 kPa) resulting in a reversal of the pCO₂ gradients across the gills and a respiratory acidosis. Fishes compensate for the acidification through metabolic compensation, by increasing the plasma HCO₃⁻ concentration. In freshwater fishes NHE2 mRNA expression has been shown to increase with hypercapnia (mummichog, Edwards *et al.*, 2005; rainbow trout, Ivanis *et al.*, 2008), while in seawater fishes NHE3 has been shown to increase (mummichog, Edwards *et al.*, 2005; medaka, Tseng *et al.*, 2013; red drum (*Sciaenops ocellatus*), Allmon and Esbaugh, 2017). In contrast the basolateral NHE1 isoform has a housekeeping role and shows a variable response from either being downregulated during periods of increased acid secretion (Claiborne *et al.*, 1999; Deigweiher *et al.*, 2008), no change (mummichog in fresh water, Edwards *et al.*, 2005), or an increase in expression (mummichog in seawater, Edwards *et al.*, 2005; red drum, Allmon and Esbaugh, 2017). In seawater, medaka exposed to hypercapnia, Liu *et al.* (2016), demonstrated an increase in acid and Cl⁻ secretion using the SIET method. Deigweiher *et al.* (2008) observed in marine eel pout (*Zoarces viviparus*) an increase in NBC mRNA levels during long-term exposure (42 days) to 10,000 µatm CO₂, and Allmon and Esbaugh (2017) observed a similar increase in red drum exposed to 30,000 µatm CO₂. High levels of hypercapnia also increase gill carbonic anhydrase activity/expression (Perry *et al.*, 2010) to support acid excretion. As might be expected, the recovery of acid–base balance is more rapid in fish in seawater because of the higher environmental HCO₃⁻ and NaCl concentrations (Iwama and Heisler, 1991).

Given Na⁺/K⁺-ATPase's central role in ion regulation in the fish gill, numerous studies have examined the effects of hypercapnia on activity and expression levels. In chronically exposed Atlantic cod (*Gadus morhua*), Melzner *et al.* (2009) demonstrated elevated activity at 0.6 kPa CO₂ (6000 µatm) for 12 months but not for the shorter exposure at a lower level of hypercapnia (4 months at 0.3 kPa (3000 µatm)). In the marine eel pout exposed to

1% CO₂ (10,000 µatm) for 42 days, Na⁺/K⁺-ATPase activity increased (Deigweiher *et al.*, 2008). However, Seidelin *et al.* (2001) found Na⁺/K⁺-ATPase activity levels of Atlantic salmon (*Salmo salar*) smolts were unaffected but mRNA levels were reduced under short-term hypercapnia (4 days, 20,000 µatm CO₂).

Marine elasmobranch fishes respond to hypercapnia in a very similar manner to teleosts. To compensate for the respiratory acidosis, they also increase plasma HCO₃⁻ levels (Heisler, 1993; Claiborne, 1998). However, the decrease in plasma Cl⁻ concentration as HCO₃⁻ concentration increases with extreme hypercapnia observed in teleost fishes has not been observed in elasmobranch fishes (reviewed by Ishimatsu *et al.*, 2005). In terms of understanding the transporters that are involved, relatively little is known. It has been shown that a metabolic acidosis (acid infusion) results in an increase in gill NHE2 and Na⁺/K⁺-ATPase expression and net acid secretion (Tresguerres *et al.*, 2005; Claiborne *et al.*, 2008). In euryhaline Atlantic stingray (*Dasyatis sabina*) and bull shark (*Carcharhinus leucas*) NHE3 is the prominent isoform expressed in fresh water although it is unresponsive to hypercapnia (Choe *et al.*, 2005; Reilly *et al.*, 2011). These results would suggest that NHE2 would be responsive to hypercapnia.

9.5 Temperature

Since fish are ectothermic poikilotherms, changes in environmental temperatures will have direct consequences for body temperatures and thus reaction and metabolic rates. Typically a 10°C increase in temperature will double reaction rates (Q₁₀ effect). Rising temperature also decreases gas solubility and pH (Colt, 1984). Blood pH in poikilothermic animals is inversely related to temperature and parallels changes of pH at neutrality (pN) with temperature (Rahn and Baumgardner, 1972; Heisler, 1993). Blood pH is maintained at a constant relative alkalinity relative to the pN. In fish, this is reflected in a decrease in plasma total CO₂ and HCO₃⁻ concentrations, and an increase in pCO₂. The strong ion difference (see Section 9.6.1) would predict that plasma Cl⁻ concentrations would be positively correlated with temperature, and indeed this is observed in fishes acclimatized to higher temperatures. In wild adult pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus*

nerka) salmon acclimatized to 13°C versus 19°C, Jeffries *et al.* (2012) observed higher plasma osmolality and $[Cl^-]$ at the higher temperature in fresh water. Similar observations have been made in European sea bass (*Dicentrarchus labrax*, Masroor *et al.*, 2018), rainbow trout (Wagner *et al.*, 1997), tilapia (*O. mossambicus*; Allanson *et al.*, 1971), and sturgeon (*Acipenser naccarii*; Cataldi *et al.*, 1998). In Arctic grayling (*Thymallus arcticus*), Cameron (1976) observed that Cl^- (and Na^+) uptake rates increased with temperature (10°C versus 17°C) which could account for the high plasma Cl^- concentrations. Presumably, this would be more costly to the animal and would be maladaptive.

9.5.1 Higher temperature increases gill permeability

In freshwater fishes, water uptake increases with acclimatization temperature with a Q_{10} of 2 (Hunn, 1982). This is reflected in a positive correlation between urine production rate and acclimatization temperature in freshwater fishes, which is necessary to compensate for the greater osmotic water load (Motais and Isaia, 1972; Hunn, 1982). Gill water permeability is known to be temperature sensitive (Motais and Isaia, 1972; Loretz, 1979). Water is hypothesized to permeate the hydrophobic plasma membrane core through transient pockets of the phospholipid hydrocarbon chains. However, Robertson and Hazel (1999) using an *ex vivo* gill system found that gill water permeability negatively correlated with acclimatization temperature in freshwater trout and tilapia. Cholesterol was implicated in gill water permeability as part of the membrane compositional changes during thermal acclimatization to preserve the fluidity of the membranes (Hazel and Williams, 1990). However, acute increases in temperature increased the initial rates of water uptake consistent with molecular disordering of the membrane. The discrepancy between *in vivo* and *ex vivo* results probably lies in the greater oxygen demand in fish at high temperatures which will require increased gill ventilation and perfusion.

9.5.2 Gill remodelling

In addition, higher water temperatures are also associated with gill remodelling in some species whereby there is a decrease in the size of an interlamellar cell mass (ILCM) that increases lamellar surface area (Sollid *et al.*, 2005; Mitrovic and Perry,

2009; Barnes *et al.*, 2014; Phuong *et al.*, 2017). The ILCM embeds the gill lamellae (gas exchange surface) within the gill filament, effectively decreasing the surface area of the gills for passive ion fluxes across the gills. Although the functional gas exchange surface area is also decreased, it is still sufficient to meet oxygen demands. As a corollary, Tzaneva *et al.* (2011) found that temperature-associated decrease in ILCM in goldfish could be significantly reduced if fish were simultaneously exposed to hyperoxic conditions. These findings indicated that the temperature-dependent ILCM changes are likely to be linked to oxygen demands and the osmo-respiratory compromise (Gilmour and Perry, 2018). In the marine ecotype of three-spine stickleback (*G. aculeatus*) in brackish water (11‰), a similar temperature-dependent decrease in ILCM (4°C versus 14°C) was observed; however, fish in fresh water (0.3‰) showed no changes with changes in temperature (Gibbons *et al.*, 2018).

Fish ionocyte size and numbers have been shown in numerous studies to change with temperature or season (e.g. Hofer *et al.*, 2000; Metz *et al.*, 2003). Adaptive changes in ionocyte mitochondria have also been observed with acclimatization temperature (Tytler and Ireland, 1995). Mitochondrial protein content, size and membrane potential all increased with acclimatization temperature in turbot larvae (*Scophthalmus maximus*).

9.5.3 Transporters

As outlined in Sections 9.2 and 9.3, Na^+/K^+ -ATPase is a key driver of ion transport in the fish gill. Sockeye salmon on their final freshwater portion of their spawning migration held at high temperatures for 24 days had lower total Na^+/K^+ -ATPase activity (V_{max} ; Crossin *et al.*, 2008) and Na^+/K^+ -ATPase protein (Atp1a1) was downregulated in salmon held at 19°C (Jeffries *et al.*, 2014). A number of other studies have also shown a negative correlation between Na^+/K^+ -ATPase V_{max} and acclimatization temperature (e.g. McCormick *et al.*, 1997; Vargas-Chacoff *et al.*, 2018). However, it should be noted that Na^+/K^+ -ATPase activity was measured under optimal conditions at 25°C to give a measure of maximal activity and the amount of enzyme present. Given the Q_{10} effect on reaction rates, it is not surprising that both activity and Atp1a1 protein levels were lower in fish acclimatized to higher temperature. Jeffries *et al.* (2014) have argued that in migrating Pacific salmon that have limited

endogenous energy stores, a significant portion of the cellular energy budget may be used to conserve energy at the cellular level with its downregulation. In two populations of Atlantic cod, acclimatization temperature (4°C versus 10°C) had no effect on gill Na⁺/K⁺-ATPase mRNA or protein levels (Michael *et al.*, 2016a). However, Michael *et al.* (2016b) found that 10°C versus 18°C acclimatization of Atlantic cod significantly reduced mRNA expression of some Na⁺/K⁺-ATPase subunits (*atp1a1*, *atp1a2* but not *atp1a3*), but not protein levels. The gill Na⁺/K⁺-ATPase activities were measured at their respective acclimatization temperatures and the 18°C acclimatization values were nearly two-fold higher than the 10°C group, revealing an uncompensated rise of Na⁺/K⁺-ATPase capacities with habitat temperature. In contrast, in the same study, Michael *et al.* (2016a) found that Na⁺/K⁺-ATPase capacities in the whiting (*Merlangius merlangus*) were lower than the two cod populations but were cold compensated (1.5 times higher Na⁺/K⁺-ATPase mRNA and protein). This may be advantageous in terms of reducing energy costs.

In a study by Metz *et al.* (2003) in crucian carp (*Carassius carassius*) acclimatized to 15°C, 22°C and 29°C for 2 months, Na⁺/K⁺-ATPase activities were measured at the different acclimatization temperatures. They found that $V_{\max}$ was lower at higher temperature but the V_{apparent} (activity measured at the acclimatization temperature) was higher. In addition, ionocytes identified by immunohistochemistry for Na⁺/K⁺-ATPase α subunit in the gills of cold-acclimatized fish were more abundant, and larger than warm-water-acclimatized fish. In contrast, in European sea bass Masroor *et al.* (2018) found no change in Na⁺/K⁺-ATPase activity (V_{apparent}) measured at acclimatization temperatures (18°C versus 26°C), and also noted an abundance of Na⁺/K⁺-ATPase-immunoreactive ionocytes in freshwater at the lower temperature.

When looking for temperature effects on other ion transporters under normocapnic conditions, Michael *et al.* (2016b) did not find any effects on gill NKCC1, NBC1, NHE1a or NHE2 in whiting or cod. However, Slc26a6, H⁺-ATPase and NHE1b mRNA expression levels were all depressed at 18°C versus 10°C. Protein levels did not show any differences with temperature under normocapnic conditions. Temperature had no effect on ECaC or NHE3 gene expression levels in different stickleback ecotypes (marine, anadromous or fresh water) (Gibbons *et al.*, 2018).

Using an acute temperature protocol to expose the estuarine fishes longfin smelt (*Spirinchus thaleichthys*) and delta smelt (*Hypomesus transpacificus*) to their upper thermal temperatures in the wild (20°C), Jeffries *et al.* (2016) detected differential expression of genes involved in ion regulation using a transcriptomics approach. For example, *aqp4* (aquaporin-4) and *trpm4* (transient receptor potential cation channel subfamily M member 4-like) were upregulated, and *kcnab3-like* (voltage-gated potassium channel subunit beta-3-like), *kcnip4* (Kv channel-interacting protein 4-like isoform X1) and *cacna1f* (voltage-dependent L-type calcium channel subunit alpha-1F-like) were downregulated. There is concern about the viability of important estuarine nursery grounds for fishes during periods of drought, and these results suggest that osmoregulatory function might be impacted at elevated temperatures.

9.6 Ocean Acidification (OA): Mild Aquatic Hypercapnia

The earlier studies looking at extreme levels of hypercapnia clearly demonstrated that fishes can compensate for the respiratory acidosis (Section 9.4.3), and that the much smaller magnitude of CO₂ change associated with OA can be dealt with. Even though the elevation of water pCO₂ with OA is less than that commonly measured in the plasma, excretion is none the less impaired and plasma pCO₂ levels increase as metabolic CO₂ accumulates. A number of studies have shown that fishes are able to compensate for OA (700–2200 $\mu\text{atm CO}_2$)-induced acid–base disturbance with either the ion transport systems already in place or by changes in mRNA or protein expression to reduce HCO₃[−] excretion, increase H⁺ secretion and/or HCO₃[−] uptake, but with species and life-history stage differences (toadfish 1900 $\mu\text{atm CO}_2$, Esbaugh *et al.*, 2012; larval medaka 1200 $\mu\text{atm CO}_2$, Tseng *et al.*, 2013; Atlantic cod 1200–2200 $\mu\text{atm CO}_2$, Michael *et al.*, 2016b; red drum 1000 $\mu\text{atm CO}_2$, Allmon and Esbaugh, 2017). In red drum no change was found in NHE1, 2, 3, NBC, cytosolic carbonic anhydrase (Cac) or H⁺-ATPase mRNA expression with 3–14 days 1000 $\mu\text{atm CO}_2$ exposure, indicating no changes at the mRNA level at least were necessary (Allmon and Esbaugh, 2017). In larval medaka exposed to 1200 $\mu\text{atm CO}_2$, mRNA levels of NHE3, NBCa and AE1a all increased as well as all ammonia transporters

(Rhag, Rhbg, Rhcg) (Tseng *et al.*, 2013). In contrast, H⁺-ATPase, CAC, NBCb and AE1b did not change significantly. In the toadfish (Esbaugh *et al.*, 2012) and Atlantic cod (Michael *et al.*, 2016b) NBC levels did not change, which contrasts with results seen with extreme hypercapnia where both gill NBC and CAC increase to defend plasma pH (Deigweiher *et al.*, 2008; Perry *et al.*, 2010).

Although Allmon and Esbaugh (2017) did not see differences in any NHE mRNA levels, in larval medaka NHE3 levels were significantly elevated at 1200 μ atm CO₂ similar to adult gill exposed to 7000 μ atm CO₂ (Tseng *et al.*, 2013). Michael *et al.* (2016b) did not measure NHE3 mRNA levels, but did not find any changes in NHE2 protein or mRNA levels. An increase in expression of apical NHE (2 or 3) would suggest involvement in acid excretion and a need for increased capacity for acid excretion. The picture of the basolateral NHE1 isoforms is less clear, which may relate to their role in housekeeping functions. NHE1a mRNA levels decreased at 2200 μ atm CO₂ but NHE1b levels increased at 1200 μ atm CO₂ (Michael *et al.*, 2016b).

The decrease in gill CAC in toadfish found by Esbaugh *et al.* (2012) has been suggested to support transepithelial uptake of HCO₃⁻ from the water rather than H⁺ excretion, since the buildup of intracellular HCO₃⁻ would not benefit apical uptake (Heuer and Grosell, 2016). This is an intriguing mechanistic response that suggests a yet-to-be-identified apical Cl⁻/HCO₃⁻ anion exchanger switches from HCO₃⁻ secretion to absorption or that another, yet-to-be-identified HCO₃⁻ uptake mechanism is employed. Esbaugh *et al.* (2012) did not find any changes in mRNA expression of the apical anion exchangers *slc26a6* or *slc26a3* and observed that AE2 (*slc4a2*), another apically expressed anion exchanger, decreased with hypercapnia suggesting its role would be for HCO₃⁻ excretion in exchange for Cl⁻ (Romero *et al.*, 2004) rather than HCO₃⁻ uptake. In agreement with findings by Esbaugh *et al.* (2012), Michael *et al.* (2016b) found *slc26a6* mRNA was significantly lower in 1200 μ atm CO₂-exposed Atlantic cod at 10°C but no differences in protein level expression were found. In freshwater fishes at least, the apical exposure of gill CCs decreases with exposure to extreme hypercapnia (Evans *et al.*, 2005). Of the four species discussed, the larval medaka appears to be more responsive to OA, which might reflect species or life-stage differences to the other studies.

The changes in gill Na⁺/K⁺-ATPase activity in response to OA are variable and not always in agreement with studies using extreme hypercapnia. No changes in gill Na⁺/K⁺-ATPase with elevated CO₂ (4 weeks 1200 μ atm or 2200 μ atm) in Atlantic cod (Michael *et al.*, 2016b) or Atlantic salmon smolts (1010 μ atm; McCormick and Regish, 2018) has been observed. In toadfish, there was only a transient decrease in gill Na⁺/K⁺-ATPase activity observed (Esbaugh *et al.*, 2012). In contrast in red drum, Esbaugh *et al.* (2016) observed an increase in branchial Na⁺/K⁺-ATPase at 1000 μ atm, which they related to a possible osmo-respiratory compromise resulting from a decrease in gill diffusion distance and increase in ventilation.

Although a respiratory response to hypercapnia is generally not as effective in compensating for a respiratory acidosis, acute hypercapnia has been shown to elicit an increase in ventilation frequency and amplitude in a number of species, albeit not universally (Gilmour, 2001). Ern and Esbaugh (2016) estimated that OA (1000 μ atm CO₂ for 24 h)-stimulated hyperventilation could attenuate the metabolic compensation by 38% in red drum. The ability to completely attenuate the metabolic response was not limited by ventilatory capacity, osmo-respiratory compromise or standard metabolic rate (SMR) and the authors suggested that there is a trade-off between the need for acid-base homeostasis and costs to ion regulation that limits the ventilatory response. Given that CO₂ excretion by the gill has an apparent diffusion limitation, Esbaugh *et al.* (2016) also found that acclimatization to OA resulted in a 32% reduction in the branchial diffusion distance and increased expression of Rhag and Rhcg1, which are two putative CO₂ channel proteins. Tseng *et al.* (2013) also observed an increase in Rhcg1 in medaka exposed to hypercapnia. Unexpectedly, Esbaugh *et al.* (2016) did not observe any significant alteration in blood chemistry in comparison with acutely challenged fish. Future work in this area is warranted given that the zebrafish has been shown to have a hyperventilatory response to low water pCO₂ levels (1 mm Hg or 1300 μ atm CO₂; Vulesevic *et al.* 2006).

The metabolic costs of ion regulation in fishes has been estimated as a minor component of SMR (reviewed by Ern *et al.*, 2014). Under severe hypercapnia (1% CO₂), the overall rates of branchial energy turnover do not change, however, the fractional costs of ion transport increase (as determined *in vitro* using ouabain, a specific inhibitor

for Na⁺/K⁺-ATPase) in Antarctic (*Gobionotothen gibberifrons* and *Notothenia coriiceps*) and temperate (*Z. viviparus*) fishes (Deigweiher *et al.*, 2010). In Atlantic cod acclimatized to 3000 μ atm or 6000 μ atm for 4–12 months, Melzner *et al.* (2009) found no effect on either metabolic or swimming performance. In the estuarine red drum, Esbaugh *et al.* (2016) did not find any effect of OA (1000 μ atm CO₂) on any measure of metabolic rate (standard (SMR) or maximal (MMR) metabolic rate, or aerobic scope (AS)) to suggest additional costs to ion and acid–base regulation.

9.6.1 Strong ion difference² – potential changes in [Cl⁻]

Tresguerres and Hamilton (2017) have raised pertinent questions surrounding the support for the link between the metabolic compensation to OA hypercapnic acidosis and Cl⁻ ion concentration changes. Exposure to OA conditions of 600–2000 μ atm CO₂ result in a 2–5 mM increase in plasma [HCO₃⁻] and complete pH recovery (see Tresguerres and Hamilton, 2017). Due to the strong ion difference, as [HCO₃⁻] increases it is predicted that [Cl⁻] should decrease proportionally. Although studies using extreme levels of hypercapnia (1% CO₂ or 10,000 μ atm and higher) have shown these corresponding changes in [Cl⁻] in teleost fishes (Ishimatsu *et al.*, 2005), studies using relevant OA conditions (Esbaugh *et al.*, 2016) or elasmobranch fishes (Heisler, 1993; Green and Jutfelt, 2014; Heinrich *et al.*, 2014) have not. This might be due to difficulty in measuring small changes in [Cl⁻] against a higher background, or alternative Cl⁻ independent HCO₃⁻ uptake mechanisms. Work is clearly needed to address the mechanisms of ion and acid–base regulation under relevant OA conditions.

9.6.2 Maladaptive intestinal HCO₃⁻ secretion

The HCO₃⁻ accumulation as part of the metabolic compensation to OA has also been found to impact the intestine of marine teleost fishes. Alkalinization of the intestinal lumen is important for precipitating imbibed calcium as CaCO₃. This precipitation provides a number of advantages: (i) it reduces calcium for absorption which is not needed; and (ii) it reduces the osmolality of the luminal fluid by up to 100 mM to aid in osmotic water absorption. The apical alkalinization mechanism is an electrogenic

Cl⁻/HCO₃⁻ exchanger (*slc26a6*), and the Cl⁻ accounts for 70% of intestinal Cl⁻ uptake. The intracellular HCO₃⁻ for exchange originates from the intracellular hydration of CO₂ catalysed by cytosolic carbonic anhydrase (CAc) and basolateral uptake of HCO₃⁻ from the extracellular fluid via a Na⁺:HCO₃⁻ cotransporter (NBC, *slc4a4*) (Whittamore, 2012; Grosell, 2014). The OA (1900 μ atm CO₂)-associated increase in pCO₂ and [HCO₃⁻] has been shown to increase intestinal HCO₃⁻ secretion by 13% in toadfish (Heuer *et al.*, 2012). Using a high range of CO₂ concentrations (5000–20,000 μ atm CO₂) Heuer and Grosell (2016) demonstrated that the increase in HCO₃⁻ secretion is reflected in an increase in the intestinal metabolic rate (8%) as measured *in vitro*. Predictions of increased CaCO₃ accumulation, however, have not been observed and fractional fluid absorption was largely unaffected (Heuer *et al.*, 2016a). Thus, although intestinal HCO₃⁻ loss is wasteful, it does not appear to negatively impact overall osmoregulation.

9.6.3 GABA_A receptor model, behaviour and ion and acid–base regulation

Although the predicted impacts of OA (700–1900 μ atm CO₂) on fish ion and acid–base physiology are generally minor given the robust capacity of fishes (reviewed by Ishimatsu *et al.*, 2008), the effects of OA on fish behaviour have yielded a number of interesting findings (reviewed by Nilsson and Lefevre, 2016; Tresguerres and Hamilton, 2017). The pioneering work by the group of Philip Munday on tropical reef fishes revealed that OA negatively impacted the response to olfactory cues for reef settlement (Munday *et al.*, 2009), and avoidance to predators (Dixson *et al.*, 2010) in orange clownfish (*Amphiprion percula*). Munday *et al.* (2010) also showed that these effects were dependent on the magnitude and duration of the CO₂ increase and that it was reversible. They also extended these findings to the damselfish (*Pomacentrus wardi*). Perhaps, unsurprisingly, these behavioural changes resulted in increased mortality in field experiments. However, it was the collaborative study with Goran Nilsson that provided the mechanistic evidence linking these behavioural changes to ion and acid–base regulation through the neuronal GABA_A receptor (Nilsson *et al.*, 2012).

The neurotransmitter γ -aminobutyric acid (GABA) and its GABA_A receptor are typically thought to be responsible for inhibitory responses

in the nervous system. Under normal conditions, during stimulation of the receptor by GABA, the receptor acts as a channel for HCO_3^- and/or Cl^- ions to enter the cell, leading to a cellular hyperpolarization, and a concomitant inhibitory response with a normal or expected behavioural response (Fig. 9.5). However, as part of the metabolic response to hypercapnic acidification, changes in extracellular and/or intracellular HCO_3^- and Cl^- concentrations are thought to reverse ion movements through the GABA_A receptor, leading instead to a depolarizing excitatory response and an altered behavioural response. In the study by Nilsson *et al.* (2012), they used the competitive GABA_A receptor antagonist gabazine to block the receptor channel and the OA associated alterations in behaviour (olfaction and lateralization (left-right turning preference)). A number of other studies have since used gabazine to produce similar results in other species from diverse environments including:

- tropical marine: damselfish (*Acanthochromis polyacanthus*, Chung *et al.*, 2014; Chivers *et al.*, 2014) and leopard coral grouper (*Plectropomus leopardus*, Munday *et al.*, 2013);
- temperate marine: three-spined stickleback (*G. aculeatus*, Jutfelt *et al.*, 2013; Lai *et al.*, 2015) and rockfish (*Sebastes diploproa*, Hamilton *et al.*, 2014); and

- fresh water: pink salmon (*O. gorbuscha*, Ou *et al.*, 2015) and catfish, (*Pangasianodon hypophthalmus*, Regan *et al.*, 2016).

These behavioural effects are not limited to teleost fishes but have been observed in elasmobranch fishes as well, for example *Mustelus canis* (Dixon *et al.*, 2015) and *Scyliorhinus canicula* (Green and Jutfelt, 2014).

In spiny damselfish exposed to 1900 μatm CO_2 , Heuer *et al.* (2016b) measured brain $[\text{HCO}_3^-]$ and intracellular pH and found changes in $[\text{HCO}_3^-]$ indicative of a metabolic compensation to the hypercapnic acidosis of OA. Using these measurements, they provided theoretical calculations of the GABA_A receptor equilibrium potential (E_{GABA}), which demonstrated that under predicted OA conditions ion movement through the GABA_A receptor may be altered explaining the observed behavioural changes. However, it has been noted that there are potential issues with these calculations (e.g. assumptions of $[\text{Cl}^-]$ and GABA_A receptor Cl^- and HCO_3^- permeability ratios and the very high pCO_2 values measured in brain of 16,000 μatm) (reviewed by Tresguerres and Hamilton, 2017).

In the three-spined stickleback, Lai *et al.* (2016) used qPCR to measure the 28 GABA_A receptor subunits and found that there was a significant effect of the high- CO_2 treatment on the mRNA expression level for the α family subunits, all showing a tendency to be more highly expressed in the OA

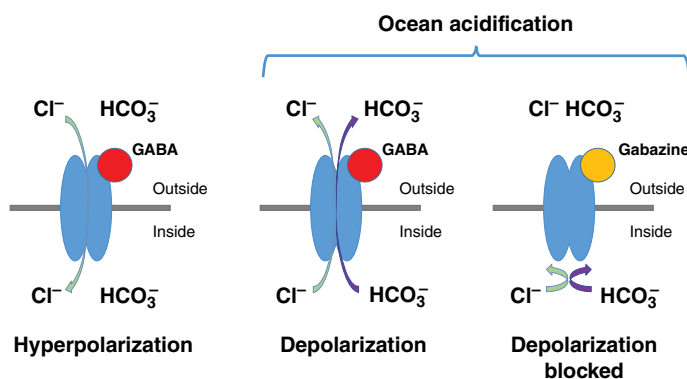


Fig. 9.5. GABA_A receptor model proposed by Nilsson *et al.* (2012) to explain behavioural changes observed during ocean acidification (OA). When gamma-aminobutyric acid (GABA) binds to the GABA_A receptor, a hyperpolarizing current results from the inflow of negatively charged ions into the neuron (inhibitory effect). During a respiratory acidosis, the fish compensates by accumulating HCO_3^- to recover pH and Cl^- concentrations would be expected to fall. Under these conditions, when GABA binds to the GABA_A receptor a depolarizing current results and there is an opposite excitatory effect. The GABA antagonist gabazine blocks the effect. (Redrawn from Nilsson and Lefevre, 2016, and Tresguerres and Hamilton, 2017.)

group. More detailed studies on the functional differences in GABA_A receptor subunits should follow. In a separate study Lai *et al.* (2017) also found that neurogenesis and neuroplasticity related genes were elevated in the three-spined stickleback, but not in the cinnamon anemonefish (*Amphiprion melanopus*) and spiny damselfish. Their results indicate differences in coping mechanisms among fish species to elevated pCO₂ levels.

Although these effects have been observed in a diverse array of fishes, the effects of OA on behaviour are by no means universal. Notably, the well-studied Atlantic cod does not have an altered behaviour (activity, boldness and lateralization) in response to near future levels of pCO₂ (1000 µatm; Jutfelt and Hedgarde, 2013, 2015). Kwan *et al.* (2017) have also found that the juvenile blacksmith (*Chromis punctipinnis*) held under either constant or oscillating CO₂-induced acidification (control 550 µatm or OA conditions 921–955 µatm CO₂) did not affect light/dark preference, inter-individual distance in a shoal or the shoal's response to a novel object. Other examples of fishes with behavioural responses insensitive to OA include the estuarine sailfin molly (*Poecilia latipinna*; Remnitz 2018), and red drum (*S. ocellatus*; Lonthair *et al.*, 2017) and reef dwelling epaulette shark (*Hemiscyllium ocellatum*; Heinrich *et al.*, 2016). Notably, these fishes live in environments that would already be experiencing high CO₂ levels. More detailed studies of these fishes are required to determine if the acid–base responses of these fishes differ.

It should be kept in mind that OA relevant changes in HCO₃⁻ and Cl⁻ in cerebral spinal fluid will also have other potential effects on neuronal function. These may be targeted through glycine receptors, network dynamics, K⁺ channels or metabolic coupling between astrocytes and neurons (Treguerres and Hamilton, 2017). In addition, CO₂-sensing peripheral neurons and/or neuroepithelial cells may also be affected (Qin *et al.*, 2010; Caprio *et al.*, 2014).

All of the above-mentioned studies on effects of OA on behaviour have involved exposures that have been measured in days to months and only on single generations and thus their relevance to end-of-century OA changes (700–1200 µatm CO₂) can be questioned. However, Welch *et al.* (2014) have found limited potential for transgenerational acclimatization in orange clownfish (*A. percula*), which suggests that genetic adaptation will be

necessary to overcome the OA effects on behaviour. In a more recent study Welch and Munday (2017) examined adaptive potential to OA transgenerational plasticity in orange clownfish and found heritability in the variation in behavioural tolerance to high CO₂.

9.6.4 Otoliths

One of the other consequences of OA is a decrease in available environmental carbonate (CO₃⁻) ions that are required by many species to form calcium carbonate (CaCO₃) for shell or skeletal elements and thus can have negative effects on growth of these structures through reduced rates of net calcification and even result in dissolution (Hoegh-Guldberg *et al.*, 2007; Doney *et al.*, 2009; Ries *et al.*, 2009). However, negative effects are not universal and may depend on a number of factors including: (i) the mechanism of pH regulation at the site of calcification; (ii) protection of the outer shell layer by an organic covering; (iii) the solubility of shell or skeletal minerals; and (iv) the extent to which the species uses photosynthetic symbionts (Ries *et al.*, 2009). Although OA results in decreased calcification of many marine invertebrate species, in marine fishes OA has actually been shown in a number of studies to increase otolith growth (Checkly *et al.*, 2009; Bignami *et al.*, 2013; Maneja *et al.*, 2013; Rossi *et al.*, 2016). Otoliths are CaCO₃ structures in the vestibular system of the inner ear of vertebrates that are important for the detection of linear acceleration and in particular gravity for orientation (Payan *et al.*, 2004). Initially, it was predicted that OA might have negative consequences on otolith growth (Ishimatsu *et al.*, 2008). Unlike in tetrapods, the otoliths of the fish inner ear are important for not only for determining equilibrium and acceleration but also for hearing (Fay and Popper, 2000). Consequently, enlarged otoliths have potentially negative consequences on larval success. In mullet (*Argyrosomus japonicus*) larvae at the settlement stage, Rossi *et al.* (2016) found that exposure to 1368 µatm CO₂ resulted in significantly larger otoliths and an avoidance response to the natural reef soundscape rather than the expected attraction which was observed in the control fish larvae (606 µatm CO₂).

Otoliths are composed of CaCO₃ (99%) primarily in the form of aragonite that is deposited on an organic matrix (< 1%) (Payan *et al.*, 2004).

In contrast to other calcification systems (e.g. bone, scales, teeth, mollusk shells and coral skeletons), otolith mineralization occurs in an acellular fluid, the endolymph, which is secreted by the inner ear epithelium, and thus calcification is dependent on endolymph chemistry. The essential components for mineralization are Ca^{2+} , CO_3^{2-} and the organic matrix. The endolymph Ca^{2+} concentrations are similar to plasma (Payan *et al.*, 2004). The ionocytes or mitochondrion-rich cells of the sacculus membrane express high levels of Na^+/K^+ -ATPase and carbonic anhydrase and have been proposed to be involved in regulating the endolymph (Mayer-Gostan *et al.*, 1997; Beier *et al.*, 2008). The proposed mechanism for Ca^{2+} supply to the endolymph has been determined using a pharmacological approach, which indicated the presence of a basolateral Ca^{2+} channel, an apical $\text{Na}^+/\text{Ca}^{2+}$ exchanger and Ca^{2+} -ATPase but the molecular identity of the transporters remains to be determined (Mugiya and Yoshida, 1995). The source of CO_3^{2-} has been shown to primarily originate from the water rather than metabolically (Tohse and Mugiya, 2008) with uptake taking place primarily across the gills (Tohse and Mugiya, 2001).

The mechanism behind the increase in otolith size with OA is most likely linked to the physiological retention of HCO_3^- in response to hypercapnia to buffer the associated acidosis (Bignami *et al.*, 2013). The result is an increase in the aragonite saturation state (Ω_{arg}) of the endolymph, thus making it easier to precipitate (Checkly *et al.*, 2009). Although OA may increase otolith growth, it does not alter otolith microchemistry (Munday *et al.*, 2011; Martino *et al.*, 2017). Since this physiological mechanism for acid-base homeostasis is maintained during exposure to hypercapnia (Welch *et al.*, 2014), the effects on otolith growth have been predicted to persist with age (Rossi *et al.*, 2016).

An additional link may be in the neurological effects of OA (Nilsson *et al.*, 2012) and the neurological control of otolith mineralization (Anken, 2006). These effects may be through changes in the chemical composition of the endolymph or through altering neurologically regulated expression of genes involved in otolith formation (Anken, 2006). Also, not all auditory impairments are associated with changes in otolith morphology. Simpson *et al.* (2011) found that juvenile clown fish (*A. percula*) exposed to OA conditions (600–900 $\mu\text{atm CO}_2$) did not avoid predator-rich daytime recordings of

a reef whereas control fish did (390 $\mu\text{atm CO}_2$) and they could find no evidence of altered otolith morphology. In addition, Munday *et al.* (2011) found no effect of OA (841 $\mu\text{atm CO}_2$) on otolith size, shape or symmetry in spiny damselfish (*A. polyacanthus*) and in other studies no effects on otolith size were found in larval Atlantic herring (*Clupea harengus*; Franke and Clemmesen, 2011), Baltic cod (*G. morhua*; Frommel *et al.*, 2012), mummichogs (Stoneman, 2013) or scup (*Stenotomus chrysops*; Perry *et al.*, 2015). Cyclical change in pCO_2 (1000 $\pm$ 300 and 1000 $\pm$ 500 μatm), which might be expected in shallow waters, did not alter otolith development in (*A. polyacanthus* or *A. percula*) under OA either (Jarrold and Munday, 2018). In species where otolith growth is maintained despite increased levels of pCO_2 , there may be physiological trade-offs in terms of reduced growth.

9.7 Combined Effects of Aquatic Hypercapnia and Warming

Few studies have examined the combined effects of OA and warming. In the Michael *et al.* (2016b) study on Atlantic cod, fish were exposed to elevated pCO_2 levels (1200 or 2200 μatm) at either 10°C or 18°C. For some gill ion transporters such as NBC1 or NHE2 there were no OA effects. For others such as *slc26a6*, *nhe1a*, *nhe1b*, *nkcc*, H^+ -ATPase or various Na^+/K^+ -ATPase α subunits, gene expression levels had a temperature-dependent response to elevated pCO_2 . Michael *et al.* (2016b) also found a pCO_2 -dependent decrease of the total RNA content of fish acclimatized to 10°C, their optimal temperature suggesting that transcriptional capacity was negatively affected. However, at warmer temperatures this effect was compensated. As a corollary, Kreiss *et al.* (2015) found that the oxygen demand for RNA and protein synthesis was lower in 10°C versus 18°C acclimatized cod exposed to elevated pCO_2 .

In Atlantic cod acclimatized to 18°C, their upper habitat temperature, exposure to OA conditions resulted in either unchanged or even decreased transcript levels of major intestinal ion transporters, in contrast to fish acclimatized to 10°C (Hu *et al.*, 2016). This possible loss in intestinal ion regulatory capacities can negatively impact organismal ion homeostasis and water balance, since HCO_3^- transport is coupled to intestinal ion and water absorption (Grosell, 2014).

9.8 Future Directions

There are a number of future avenues of research to be pursued regarding the impacts of climate change on fish ion regulation.

1. Multi-generation studies are completely lacking. However, we know from the study of Welch *et al.* (2014) that the effects of OA on behaviour are transgenerational.
2. In order to understand the link between OA and fish behaviour, more work is required on the neuronal GABA_A receptor Cl⁻/HCO₃⁻ permeability properties.
3. Why does OA affect behaviour in some fish but not in others? Is this due to differences in their GABA_A receptor or acid–base regulatory strategies?
4. The impacts of climate change on ion regulation in non-teleost fish species should be investigated. While there are a few studies on elasmobranch and chondrosteian fishes in which we can infer ion and acid–base regulatory changes, none directly addresses ion regulatory impacts. The transporters involved in acid–base regulation in response to hypercapnia have also not been elucidated. We are also unaware of any studies on any cyclostomes (lamprey).
5. What is the mechanism of apical HCO₃⁻ uptake in fish facing OA levels of aquatic hypercapnia? Existing evidence would suggest an apical Cl⁻/HCO₃⁻ exchange mechanism.
6. The mechanisms of otolith saccule ion regulation should be revisited. The molecular identification of the ion transporters at play in ion and acid–base regulation remains largely unresolved.

Notes

¹ 1 torr (mm Hg) = 0.1333 kPa = 1316 μatm.

² According to the laws of electroneutrality, the sum of strong cations (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺) must equal strong anions (Cl⁻ + lactate⁻) and the strong ion difference is the sum of the weak cations (HCO₃⁻, proteins, PO₄³⁻).

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10 Excess Dissolved Gases including Gas Bubble Disease

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10.1 Introduction

The historical baseline of carbon dioxide (CO₂) in the atmosphere is near 260 µatm, and it depends on the representative concentrations pathways (RCPs) selected (IPCC, 2013a). The concentration of CO₂ in 2100 was estimated to range from 421 µatm to 936 µatm. This increase will result in increased dissolved CO₂ in fresh and seawaters with corresponding reduction in pH. 'Excess gas' may also refer to cases where the partial pressure of a gas in the liquid phase is in excess of its partial pressure in the gas phase. This is 'gas supersaturation' and can be computed for individual gases or for the sum of all dissolved gases. While gas supersaturation is a non-equilibrium condition, the kinetics of gas transfer are slow and equilibrium gas concentrations are rarely found. Even though the concentration of CO₂ in water may be elevated, it may not be supersaturated with respect to the ambient gas concentration.

Gas supersaturation is produced by physical, chemical and biological processes. In hydroelectric systems, gas supersaturation can be produced by air entrainment in spillways or air vented into turbines (Weitkamp and Katz, 1980). In aquaculture systems, gas supersaturation commonly results from air entrainment on the suction side of pumps or heating of water in closed systems. Gas supersaturation can result in the development of gas

bubble disease (GBD) or gas bubble trauma in fish. The development of GBD depends on the level of total gas pressure and the supersaturation of a single gas may not result in GBD. At 20°C (barometric pressure = 760 mm Hg), the partial pressure of oxygen, nitrogen, argon and carbon dioxide and vapour pressure of water in fresh water are 155.5 mm Hg, 579.7 mm Hg, 6.9 mm Hg, 0.3 mm Hg and 17.5 mm Hg, respectively (Colt, 2012). Consequently, oxygen and nitrogen dominate gas supersaturation impacts. GBD in fish is characterized by the development of gas bubbles on the fins and body surfaces, and within the gills, lateral line, blood vessels and eyes. The disease has been documented for a wide variety of fish, crustaceans and molluscs under warm- and cold-water conditions. High levels of photosynthesis increase dissolved oxygen (DO) which may cause GBD (Woodbury, 1941) but the disease is usually due to the addition of oxygen and heating on ambient dissolved gases.

The carbonate system (carbon dioxide gas (CO₂), bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻)) is the main buffer system in fresh water, seawater and fish blood. The carbonate alkalinity of seawater is very stable (about 2.2–2.3 mM), resulting in a stable pH around 8.0. The fresh water alkalinity ranges from low levels in soft water (0.03–0.04 mM) to high levels in bicarbonate-rich hard water, which can be higher than seawater. The atmospheric CO₂ concentration is increasing and this

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results in increased partial pressure of CO_2 in fresh water and seawater. In seawater the reduction in pH is estimated to be 0.7–0.8 pH units by the year 2300 (Caldeira and Wickett, 2003). In soft fresh water, the pH may easily drop below pH 6, resulting in toxic aluminium (Al) forms and increased carbon dioxide levels will add to problems with acid rain. Black water rivers in the tropics also have low ionic content and low bicarbonate levels and these are comparable to soft water. Increased partial pressure of CO_2 is defined as environmental hypercapnia (Heisler, 1984, 1986). There is the same general physiological response to environmental hypercapnia for teleost fish and there will be a change in fish plasma composition. The general response in fish to an elevated bicarbonate concentration is reduced plasma chloride as a result of branchial HCO_3^- uptake (Esbaugh *et al.*, 2012) and chloride extrusion. There seems to be a dose-dependent relationship at low environmental CO_2 concentrations. According to Ishimatsu *et al.* (2005), Cl^- decreases with a nearly equimolar increase in HCO_3^- in marine teleosts under a low level of hypercapnia (8–16 mm Hg). The increased bicarbonate concentration can have many anatomical and physiological consequences in fish, some of which are known and some unknown (Esbaugh *et al.*, 2005). Elasmobranchs exposed to hypercapnia have less change in plasma chloride compared with teleosts, indicating different acid–base regulatory mechanisms (Ishimatsu *et al.*, 2005).

During short-term hypercapnia, blood pH is reduced and oxygen uptake may also be reduced (Bohr effect). However, pH is generally increased to a control level or slightly above the control level by the transfer of acid–base relevant ions with the water within 3–24 h (Heisler, 1984, 1986). Nephrocalcinosis, reduced growth and reduced condition factor also occur during long-term environmental hypercapnia (Smart, 1981). Fish exposed to environmental hypercapnia have gross nephrocalcinosis and increased urinary pH (Eddy *et al.*, 1979). Hyperoxia is water oxygen saturation above 100%; it reduces gill ventilation and therefore the plasma partial pressure of carbon dioxide (pCO_2) and bicarbonate concentrations are increased. The condition resembles hypercapnia regarding acid–base balance in fish.

In teleosts, the metabolic rate is dependent on temperature and body size. For resting metabolism, a typical tropical fish at 30°C requires approximately six times more oxygen than a polar fish at

0°C (Clarke and Johnston, 1999). The respiratory quotient (RQ) depends more on the DO than temperature (Kutty, 1968). At high fish density, such as in cage culture or fish shoals, increasing temperature as with climate change will contribute to risky conditions due to decreasing DO solubility, hypoxia and enhanced CO_2 concentration. Higher levels of CO_2 reduce oxygen uptake in fish (Bohr effect, Root effect) as demonstrated by Basu (1959). However, the levels Basu (1959) used in his experiments were probably higher than levels caused by future ocean acidification. Fish exposed to elevated water temperatures (e.g. from climate change) can face an ‘oxygen squeeze’ where the decreased supply of oxygen cannot meet the increased demand (Ficke *et al.*, 2007).

In a review, Matear and Hirst (2003) predicted a 4–7% decline in DO in the ocean at the end of this century and suggested global warming might eventually drive the deep ocean anoxic. Growth of phytoplankton is temperature dependent (e.g. Eppley, 1972) and higher temperatures generally contribute to increased frequency, higher peak and longer algal blooms (Raven and Geider, 1988; Pearl and Huisman, 2008). DO in the surface water often fluctuates between saturation deficit at night/morning and supersaturation in the afternoon, and during blooms DO amplitudes are maximized. At higher temperature, marine dinoflagellates may reach daily amplitudes ranging from 3 mg/l to 5 mg/l (Prézélin *et al.*, 1977).

Aerobic respiration by algae, plants, micro-organisms and fish decrease DO in the water column, especially at night when photosynthesis stops. DO concentrations of 5 mg/l or more are acceptable for most aquatic organisms and concentrations below 2–3 mg/l are considered lethal (see Ficke *et al.*, 2007). Critical DO limits for cold-water species are 2.5 mg/l (e.g. salmonids) and 3.0 mg/l for warm-water species (carps, smallmouth bass) (US EPA, 1986).

The disorders relevant to fresh water and ocean acidification are hypercapnia, hyperoxia, GBD and also effects of metal toxicity caused by acidification of fresh water.

10.2 Diagnosis of the Disorders

10.2.1 Hypercapnia

Physiological measurements include plasma pCO_2 and HCO_3^- , while plasma pH remains normal or

slightly elevated. There is an equimolar decrease in plasma Cl^- relative to the increase in bicarbonate. Red blood cell counts are normally not influenced by high CO_2 concentration. However, erythrocyte volume may decline and erythrocyte haemoglobin may increase in Atlantic salmon (*Salmo salar*) parr exposed to increased carbon dioxide partial pressures (Fivelstad *et al.*, 2007).

Findings from short-term and long-term experiments on hypercapnia include increased adrenaline levels (Perry *et al.*, 1986b), hyperventilation (Janson and Randall, 1975; Smith and Jones, 1982; Fivelstad *et al.*, 1999; Hosfeld *et al.*, 2008), reduced branchial chloride influx rates (Perry *et al.*, 1986a; Goss *et al.*, 1994) and reduced plasma chloride (Lloyd and White, 1967; Eddy *et al.*, 1977; Fivelstad *et al.*, 1999, 2003a, b). Elevated plasma cortisol occurs during acute CO_2 exposure (Petochi *et al.*, 2011).

Nephrocalcinosis ('kidney stones') is calcium phosphate deposits in the excretory tissues of the kidney and is also found in the stomach wall (Smart *et al.*, 1979). The kidney calculi may cause dilation of tubule lumina and tissue death and the functional kidney can be destroyed. Under farming conditions, the deposits can be a result of high levels of dissolved CO_2 in fresh and seawaters (Smart *et al.*, 1979; Fivelstad *et al.*, 2003a, b, 2017). Increased urinary pH has been associated with calcium deposits in the kidney. Increased nephrocalcinosis and contents of calcium in the kidney have been found after 2 months of exposure to increasing water CO_2 levels in fresh water (Fivelstad *et al.*, 2003b).

Exposure to hypercapnia may result in an increase in trabeculae volume and a higher rate of bone remodelling in freshwater fish and subsequently a higher content of bone ash (Gil *et al.*, 2006). The mechanical strength of the bones may be influenced by chemical change(s) in the bones. Higher prevalence of polymorph calcium carbonate (vaterite) in sagittal otoliths is associated with hearing impairment in farmed salmon (Reimer *et al.*, 2016). Further research is needed as it may be related to hypercapnia. Reduced hearing may have pronounced survival effects in wild fish and in escaped farmed fish.

Atlantic salmon juveniles (33 g) exposed to elevated freshwater hypercapnia (up to 33 mg/l) at a constant elevated water temperature of 15°C, showed reduced white muscle buffering capacity. Similar to pelagic fish such as scombroids, Atlantic salmon seem to rely on histidine (His) and His-related compounds to buffer protons produced by high anaerobic activity in white muscles (Suzuki

et al., 1987, 1990). The dipeptide anserine (Ans) is the major free amino acid in Atlantic salmon muscles (Andersen *et al.*, 2016) and is directly related to the buffering capacity as measured by OH-titration. On a molar basis, masu salmon (*Oncorhynchus masou*) parr increased muscle Ans levels (40%) at the expense of free His levels (decreased 50%) during the parr-smolt transformation (Ogata *et al.*, 1998). Similarly, increase in Ans in Atlantic salmon during parr-smolt transformation provided a sufficiently high dietary His level (Breck *et al.*, 2005). At the physiological pH range of the cold-water fish muscle, the buffering capacity of the His dipeptides are very high and higher than that of free His, and the capacity seems to be constant within the rearing temperature range in normal salmonid farming (Abe and Okuma, 1991). Thus, sufficient imidazole compounds seem to be important in coping with hypercapnia and acidosis, especially since the buffering capacity of these imidazole compounds are less influenced by temperature. The exposure to high CO_2 concentrations also influenced the occurrence of oxidation products in the liver, suggesting that respiratory acidosis is followed by metabolic changes and oxidative stress (see Chapter 6, this volume). This resembles the time-limited coping mechanisms at more extreme temperatures, with anaerobic metabolism, and protection by antioxidation and heat shock proteins (Pörtner, 2002).

Cataracts in farmed fish are a sensitive indicator of nutritional (Bjerkås *et al.*, 2006) and production-related disorders (Waagbø, 2008). Atlantic salmon parr exposed to three fresh water oxygenation regimes (95%, 112% or 125% saturation), with or without supplementation of CO_2 (2 mg/l and 18 mg/l) for 6 weeks were followed up with cataract inspections and tissue samplings after the freshwater period and after 6 more weeks in a common seawater regime. Fish showed a growth-related cataract development in seawater, mostly in fish previously exposed to normoxic and hyperoxic freshwater conditions. Reduced protection of the fish lens against external oxidative stress was indicated by early molecular markers such as mRNA expressions of heat shock protein 70 (HSP70) and antioxidant enzymes (Waagbø *et al.*, 2008). Atlantic salmon smolts raised at normal seawater temperature are in need of elevated dietary histidine to prevent cataract development (Remø *et al.*, 2014) and both elevated freshwater and seawater temperatures (stable 16°C) increased the risk for cataract development compared with fish reared at 10°C (Sambras *et al.*, 2017).

Periods of elevated water temperatures and gas concentrations affect the stress physiology and welfare of farmed fish, visualized as elevated plasma cortisol levels (Segner *et al.*, 2011). Hydromineral changes observed in hypercapnia (Fivelstad, 2003b) may be compounded by the acute or chronic stress responses and vice versa. Physiological mechanisms of regaining homeostasis and coping may, however, differ considerably. Segner *et al.* (2011) presented health and disease parameters that can be used as welfare indicators, including haematology, clinical chemistry, plasma cortisol, immunological markers and expressions of stress-related genes. An example of the selective use of markers at different organization levels was recently given by Waagbø *et al.* (2017) who examined starvation and stress in adult Atlantic salmon at low temperature.

Climate change such as increased water temperatures and carbon dioxide level affect the welfare of farmed fish. Studies also show a potential to mitigate the climate impacts using climate-friendly feeds.

10.2.2 Low pH/high CO₂ and Al

Increased Al concentrations on the gills (strongly dependent on pH) is associated with increased mucus production and hypertrophy and hyperplasia of gill epithelium. There is a rapid reduction of sodium and chloride levels in the plasma. During acute exposure plasma chloride may be lowered to 80–90 mM before a fish dies. Respiration frequency may be twice the normal rate and the fish coughs more frequently than normal.

While negative effects related to Al are found in Norwegian rivers (pH lower than 6), there are less or no effects in Canadian rivers with pH from 5.0 to 5.5 (Lacroix, 1989). In Nova Scotia, Canada, the toxic concentrations of inorganic monomeric Al in rivers are reduced by organic contents. In experimental studies Al-related toxicity was eliminated by complexing Al with citrate – there were few or no physiological effects, growth was good and mortality low (Fivelstad *et al.*, 2004).

10.2.3 Gas bubble disease (GBD)

According to Schiewe and Weber (1976), GBD occurs when gas bubbles cover more than 15% of the lateral line in juvenile steelhead trout (*Oncorhynchus mykiss*). However, scattered bubbles may occur on fish when the water is not supersaturated. Bubbles which fill the scale pockets of

the lateral line may reduce the ability of the sensory systems to respond to stimuli (e.g. predators). Bubbles or emboli may also be in gill blood vessels, and bubbles in the gills may cause death (Weitkamp and Katz, 1980). Dawley *et al.* (1976) found increased prevalence of emboli in branchial arteries and gill filaments of dead fish compared with live fish. According to Weitkamp (1976) bubbles on the head, opercula, jaws and mouth occurred only after the appearance of bubbles in the skin.

Adult salmonids may have bubbles in the roof of the mouth and exophthalmia (Wood, 1968). Abnormal behaviour occurs and a non-specific sign is a loss in equilibrium. Herring (*Clupea harengus*) larvae may develop gas bubbles in their guts, resulting in death (Dannevig and Dannevig, 1950). Trout fry reared in supersaturated water may rise to the surface, lifted by gas bubbles on their surface, and bubbles in their mouth may cause suffocation (Peterson, 1971). In salmonid sac fry, bubbles between the yolk sac and the perivitelline membrane may cause the fry to swim head up (Stroud *et al.*, 1975). Generally, eggs appear to be resistant to gas supersaturation, but not always.

Commercially reared Atlantic halibut (*Hippoglossus hippoglossus*) juveniles reared at two densities (12.5 kg/m² and 18.2 kg/m²) experienced high prevalence of eye damage, but prevalence was higher in the tanks with lowest density (Remø *et al.*, 2011). Formation of gas bubbles and eye damage seemed to be related to the hyperoxygenated inlet seawater (151% oxygen) and high water flow used in the in-house production regime to promote growth.

10.2.4 Hyperoxia

The physiological response from hyperoxia resembles the response from hypercapnia. The difference is that reduced gill respiration causes increased pCO₂ in blood, and the following pH reduction in plasma is compensated by increased bicarbonate (Heisler, 1984), as is also the case during hypercapnia. Generally, high levels of hyperoxia causes GBD (see Section 10.2.3), while moderate levels may improve growth (Hosfeld *et al.*, 2008; Lemarie *et al.*, 2011). In fish farming it is possible to add an extra partial pressure of oxygen without increasing the total gas pressure in the water, and by getting rid of an equivalent partial pressure of nitrogen. It is difficult to distinguish between physiological effects of hyperoxia and GBD. Edsall and Smith (1990) found reduced haematocrit and haemoglobin levels in rainbow trout (*Oncorhynchus mykiss*) exposed to 180%

oxygen saturation and low total gas pressure, which may be a result of low demand for oxygen transport capability under hyperoxic conditions.

Oxygenation is used in tank-based farming systems to promote growth and welfare at elevated farming densities. Production protocols including oxygenation have been developed for many species, and considers growth performance, feed and feeding efficiency, and fish welfare (Caldwell and Hinshaw, 1995). Despite constant control, both chronic and short incidences of hyperoxia may generate oxidative stress in the fish and the question is how the fish cope in the short and long term. Oxidative stress in the tissues is met by an array of endogenous and exogenous antioxidant compounds and antioxidant enzymes (Hamre *et al.*, 2010, 2017). This implies that the integrated antioxidant system can be improved to some extent by dietary means, like excess supplementation of antioxidant vitamins such as vitamins E and C (Lygren *et al.*, 2000; Waagbø, 2008; Hamre *et al.*, 2010, 2017). As an example, groups of Atlantic salmon smolt fed three levels of vitamin E (40 mg/kg, 300 mg/kg and 1100 mg/kg) were exposed to two levels of oxygen saturation, normal saturation at 90% and hypersaturation at 150% (Lygren *et al.*, 2000). Hyperoxic fish showed elevated mortalities and development of gas bubbles under the skin and around the gills. Liver vitamin E status reflected the dietary vitamin E levels, and it was considerably reduced by hyperoxic conditions. Similarly, other antioxidants such as liver vitamin C, superoxide dismutase activity, glutathione peroxidase activity and total mercaptans were reduced concomitantly to an increased oxidative status. This study did not discriminate between effects from hyperoxia and GBD. The lack of such discrimination is a challenge also in further research.

10.3 Field and/or Experimental Studies, and Projection(s) on Future Trends as the Climate Changes

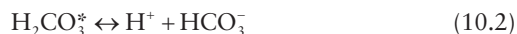
10.3.1 CO₂, alkalinity and pH/Al and future trends

Carbonic acid is formed when CO₂ dissolves in water:



Since only a minor part of the CO_{2(aq)} is converted to H₂CO₃, and because it is difficult to distinguish between CO_{2(aq)} and H₂CO₃ analytically, a hypothetical compound H₂CO₃^{*} has been suggested (Gebauer

et al., 2005). H₂CO₃^{*} is the sum of CO_{2(aq)} and H₂CO₃. The equilibrium equations are:



The sum of H₂CO₃^{*}, HCO₃⁻ and CO₃²⁻ is defined as total carbonate:

$$C_T = [\text{H}_2\text{CO}_3^*] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (10.4)$$

The fractions of total carbonate as H₂CO₃^{*}, HCO₃⁻ and CO₃²⁻ are given by α₀, α₁ and α₂, respectively (Equations 10.5–10.7):

$$\alpha_0 = \frac{[\text{H}_2\text{CO}_3^*]}{C_T} = \left(1 + \frac{K_1}{[\text{H}^+]} + \frac{K_1 K_2}{[\text{H}^+]^2} \right)^{-1} \quad (10.5)$$

$$\alpha_1 = \frac{[\text{HCO}_3^-]}{C_T} = \left(\frac{[\text{H}^+]}{K_1} + 1 + \frac{K_2}{[\text{H}^+]} \right)^{-1} \quad (10.6)$$

$$\alpha_2 = \frac{[\text{CO}_3^{2-}]}{C_T} = \left(\frac{[\text{H}^+]^2}{K_1 K_2} + \frac{[\text{H}^+]}{K_2} + 1 \right)^{-1} \quad (10.7)$$

in which K₁ is the first acidic constant (H₂CO₃^{*} ↔ H⁺ + HCO₃⁻) and K₂ is the second acidic constant (HCO₃⁻ ↔ H⁺ + CO₃²⁻).

In fresh water, the equilibrium constants are dependent on temperature. For seawater there are two different sets of equilibrium constants related to two different pH scales (Mehrbach *et al.*, 1973; Millero *et al.*, 2002). In general, when total carbonate is measured, CO₂ is obtained by multiplying α₀ with the carbonate concentration, using appropriate equilibrium constants in agreement with the pH scale used. Therefore, the pH scale used should always be defined in seawater studies.

Alkalinity is the capacity of the water to neutralize acid (Millero, 1996; Stumm and Morgan, 1996). For fresh water alkalinity can be written as:

$$\text{Alkalinity} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+] \quad (10.8)$$

For seawater systems and for recirculation aquaculture systems the terms to be added are (Blancheton *et al.*, 2007):

$$\begin{aligned} \text{Alkalinity}_{\text{extra}} = & [\text{B}(\text{OH})_4^-] + [\text{NH}_3] \\ & + [\text{SiO}(\text{OH})_3^-] + [\text{HPO}_4^{2-}] \\ & + 2[\text{PO}_4^{3-}] - [\text{H}_3\text{PO}_4] \end{aligned} \quad (10.9)$$

Generally, it is the same chemical relationship regarding pH and $\text{CO}_{2(\text{aq})}$ for the water in a fish tank and in fresh water and seawater. When CO_2 accumulates in water the pH-drop is dependent on the alkalinity (Fig. 10.1), and for seawater the alkalinity is higher (about 2.3 mM, see Fig. 10.2) and the equilibrium constants are influenced by the ionic strength.

Most of the Norwegian fish farms have low alkalinity in their freshwater supply (Kristensen *et al.*, 2009). Alkalinity of fresh water can vary by a magnitude of 1:100 from about 0.02 mM (soft water) to 2 mM (hard water) primarily due to increasing concentrations of calcium and magnesium (Gray, 2010). In a comprehensive survey of inlet water to salmon hatcheries an average alkalinity of 0.09 ± 0.07 mM and 0.73 ± 0.52 mM were measured in Norway and Chile, respectively (Kristensen *et al.*, 2009). Soft fresh water is vulnerable to acidification caused by anthropogenic CO_2 (Molot *et al.*, 1989; Bulger *et al.*, 1993). The water also contains aluminium, because Al leaches from the soil because of acid precipitation (Jensen and Leivestad, 1989). The toxicity of Al is pH dependent with the highest toxicity below pH 6.0 and above pH 7.0. Because of the pH-drop in the water the toxic forms of Al may become remobilized both in fish tanks and in

nature. Therefore, Al has to be considered in all CO_2 freshwater experiments.

The main change in pH may occur between pH 7 and pH 5.5 in fresh water (Fig. 10.1) depending on alkalinity and prior exposure to acid rain. In seawater alkalinity is rather stable, mainly in the range 2.0–2.4 mM and the reduction of pH is smaller (Fig. 10.2). In seawater, the reduction of pH is calculated to be 0.7–0.8 pH units by the year 2300 (Caldeira and Wickett, 2003).

Experiments in low alkalinity fresh water (soft water)

Three different experiments were performed on Atlantic salmon smolts (40–80 g) in fresh water at Western Norway University of Applied Sciences. The water was soft (conductivity 24–32 $\mu\text{S}/\text{cm}$; calcium 0.4–0.9 mg/l, chloride 6–17 mg/l, sodium 3–5 mg/l), acidic (about pH 5) and contained total Al ($\approx 130 \mu\text{g}/\text{l}$; $\approx 4.8 \mu\text{M}$). The water was neutralized to pH 7.0–7.2 in a header tank, thereafter the water passed through mixing tanks before entering the fish tanks. The pH in the experiments ranged from 5.5 to 6.6.

The first experiment studied the combined effects of carbon dioxide, reduction in pH and the changes

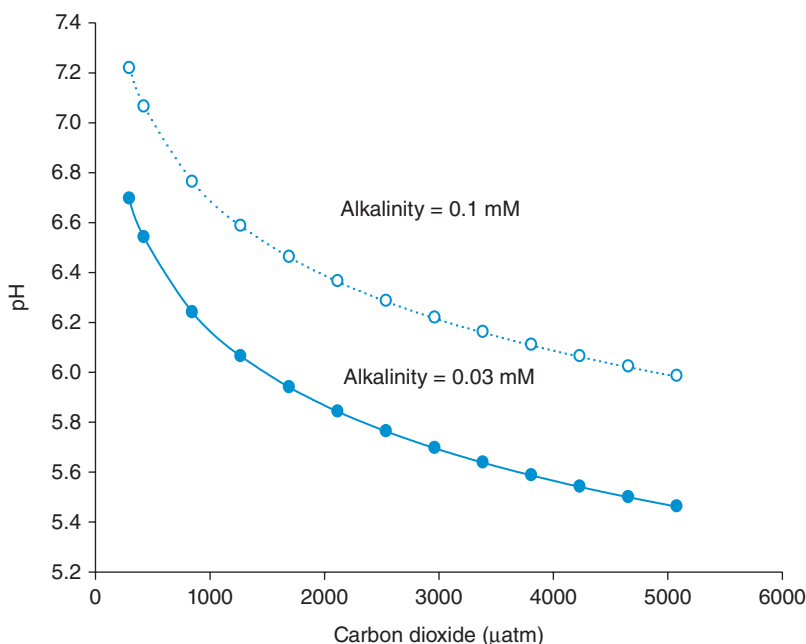


Fig. 10.1. The theoretical relationship between carbon dioxide partial pressure and pH level in fresh water. Temperature is 10°C and carbonate alkalinity is 0.1 mM and 0.03 mM. (Based on the model of Sanni and Forsberg, 1996.)

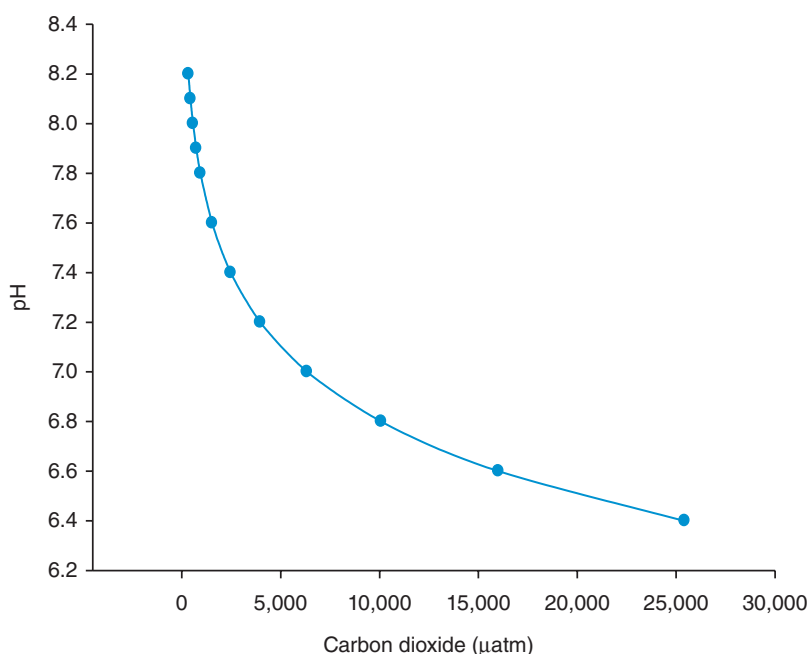


Fig. 10.2. The theoretical relationship between carbon dioxide partial pressure and pH level in seawater (34‰). Temperature is 10°C and carbonate alkalinity is 2.3 mM. (Based on an Excel spreadsheet developed by Raul Piedrahita (University of California, Davis, 2016, personal communication) and based mainly on US DOE, 1994.)

in Al chemistry (combinations: pH 6.6 and 2 mg/l CO₂; pH 6.0 and 9 mg/l CO₂; pH 5.7 and 19 mg/l CO₂) (Fivelstad *et al.*, 2003a). The alkalinity was 0.09 mM.

Hyperventilation and coughing were detected within 24 h, while significant increased glucose and plasma cortisol levels were measured after 2 days ($P < 0.05$). All measured parameters were 'influenced' and heavy mortality occurred after about 2 weeks. An association between gill Al accumulation and gill lesions were found. The lesions were mainly chloride cell hyperplasia and adhesion of filaments in water containing Al in the pH interval of 5.4–5.8. Gill lesions related to Al toxicity have been found in earlier studies (Karlsson-Norrgren *et al.*, 1986; Fischer-Scherl and Hoffman, 1988; Smith and Haynes, 1995).

Accumulated mortality in the high CO₂ group was 42% after 25 days and the experiment was discontinued for ethical reasons. There was a five-fold increase in gill Al accumulation at pH 6.0 compared with pH 6.6 (Fig. 10.3). However, the experiment did not discriminate between effects of pH alone, effects of CO₂ and Al. Further

investigations were needed to study the effects of pH alone and effects of pH and CO₂ on Atlantic salmon smolts.

In the second experiment, Al was complexed by tri-sodium citrate and pH was reduced by diluted H₂SO₄ to the same pH range as in the first experiment. Growth was good, mortality was low in all groups, and the physiological alterations were minimal (Fivelstad *et al.*, 2004). In the third experiment (Fivelstad *et al.*, unpublished) Al was also complexed by tri-sodium citrate and pH was reduced by addition of carbon dioxide. In the medium and high carbon dioxide groups, mean weight and condition factor were significantly reduced after 34 days compared with the control group ($P < 0.05$). At the end of the freshwater period (day 56), mean weight and condition factor were significantly reduced only for the high group. During the freshwater period, the specific growth rate (SGR) of the high carbon dioxide group was lower than that of the medium and the control group. At the end of the seawater period, there were no significant differences in weight and condition factor among the groups.

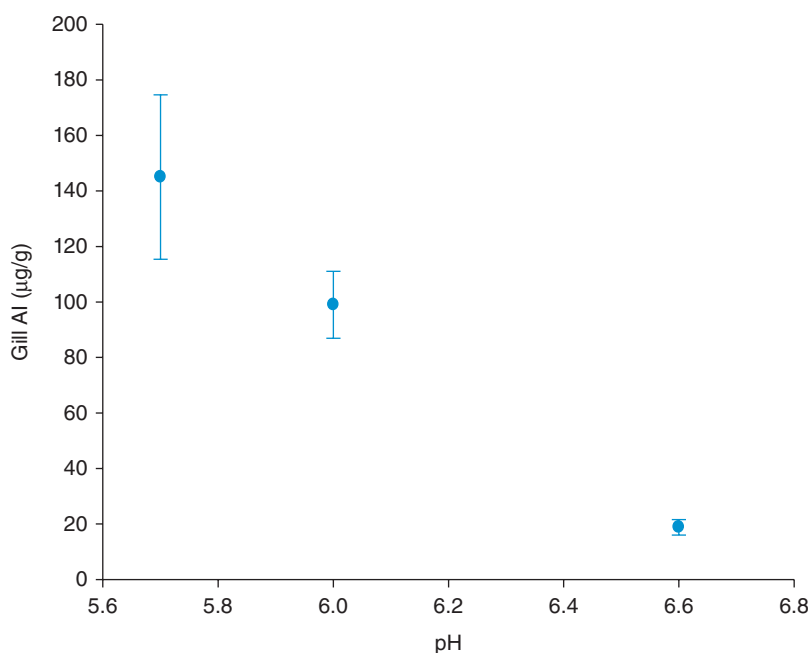


Fig. 10.3. Accumulation of aluminum in the gills of Atlantic salmon smolts for the control group (pH 6.6 and 2 mg/l CO_2), the medium carbon dioxide group (pH 6.0 and 9 mg/l CO_2) and the high carbon dioxide group (pH 5.7 and 19 mg/l CO_2). Means and SD are given. (Reproduced from Fivelstad *et al.*, 2003a with permission from Elsevier.)

These three experiments performed in the same pH range, showed that an accumulation of water CO_2 , reduction in water pH and the changes in Al chemistry may be detrimental to Atlantic salmon smolts, even at low Al concentrations. Consequently, even though pH as a single factor may not have toxic effects, and the combination of moderate CO_2 and low pH only have minor effects on salmon smolts, low concentrations of toxic Al should be avoided.

In the third experiment, significant effects on the K factor (the relationship between the weight in grammes (W) and length in centimetres (L) of a fish, $K = 100 W(L^{-3})$) was found after 1 month's exposure of Atlantic salmon smolts (45 g; pH 6.0–6.2) to 2.7 mm Hg pCO_2 (3553 μatm) which disappeared after 2 months. Gil *et al.* (2006) found significant effects on Atlantic salmon parr (10 g) after 1 month's exposure to about 3.6 mm Hg (4737 μatm ; pH 6.2; 13°C). Graff *et al.* (2002) observed significant effects in parr-smolt (35 g) exposed to 3.2 mm Hg (4211 μatm) on SGR, however, in this study influence from Al was not eliminated.

Experiments with high alkalinity fresh water

Smart *et al.* (1979) exposed rainbow trout to three CO_2 concentrations (12 mg/l, 24 mg/l and 55 mg/l) for 275 days. The experimental set-up also comprised water phosphate concentration and mineral content in the diet. Histopathological examination at the end of the experiment showed lesions in the stomach and kidney. The kidney concentrations of Ca, Mg and phosphate increased with increasing CO_2 concentrations in the water. Ureteral deposits were composed of calcium phosphate, as badly crystallized apatite and well-crystallized brushite. The trout in the experiment survived and grew even though their kidneys were severely damaged. However, Fivelstad *et al.* (1999, 2003a, b) showed that Atlantic salmon smolts had reduced growth and increased incidence of nephrocalcinosis in the high CO_2 groups. Long-term CO_2 toxicity on Atlantic salmon parr were studied at 5°C and 15°C (Fivelstad *et al.*, 2007). The experiment showed that CO_2 had highest impact on parr at the low temperature, and the effects included reduced

growth and alteration in haematological parameters. The CO_2 partial pressures in the studies of Fivelstad *et al.* (1999, 2003a, b, 2007) were higher than the most relevant range for CO_2 -induced water acidification which is considered to be 450–2000 μatm .

Recently, Ou *et al.* (2015) studied the responses of pink salmon (*Oncorhynchus gorbuscha*) to CO_2 -induced acidification in hard fresh water. Eyed embryos were exposed to constant CO_2 levels of 450 μatm , 1000 μatm and 2000 μatm and diurnal fluctuations of 450–2000 μatm . Dose-dependent reduction in growth, yolk-to-tissue conversion and maximal oxygen uptake capacity were found. The experimental set-up also included three different seawater treatments (constant 450 μatm and 1600 μatm , and diurnal variation of 450–1600 μatm). Effects on olfactory responses and behaviour also

seemed to be dose dependent. After seawater transfer, growth rates were significantly affected by CO_2 .

In both fresh water and seawater, plasma pCO_2 increases linearly as a function of pCO_2 in the water (Fig. 10.4) and there is a decrease in plasma chloride Cl^- with a nearly equimolar increase in HCO_3^- (Fig. 10.5).

Carbon dioxide/pH receptors and normal brain function

CO_2/pH receptors have been demonstrated in carp, Japanese eel (*Anguilla japonica*) and rainbow trout (Konishi *et al.*, 1969; Yoshii *et al.*, 1980; Yamashita *et al.*, 1989). Thresholds for these receptors are low, 0.5 mm Hg for eel and 0.6 mm Hg for rainbow trout. According to Perry and Gilmore (2002) fish also have branchial CO_2 receptors, participating in

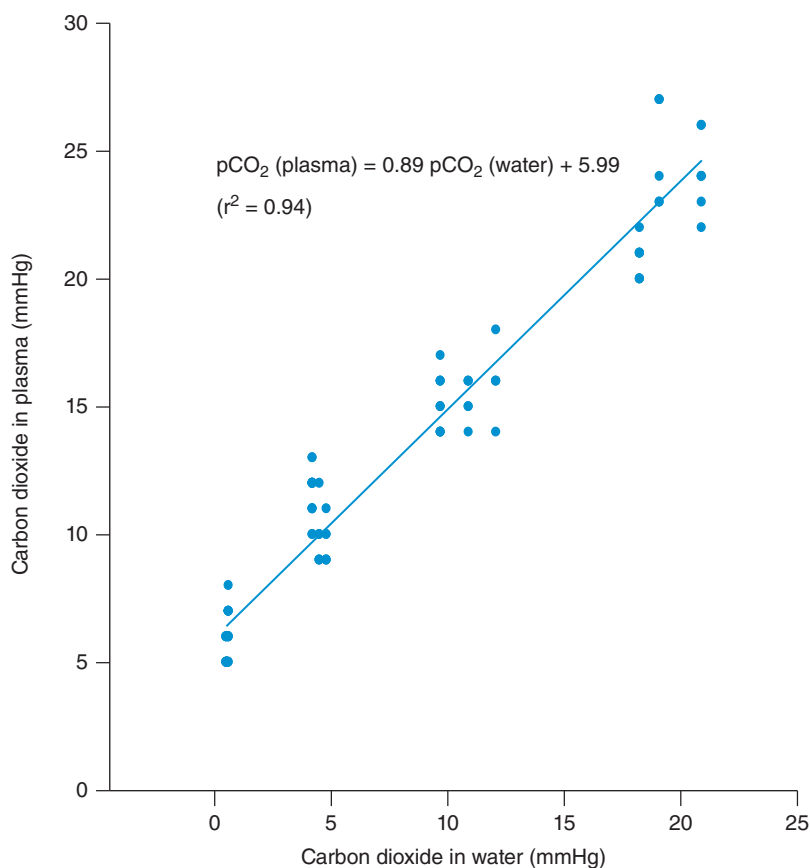


Fig. 10.4. Relationship between the partial pressure for carbon dioxide in the water and the partial pressure in the blood plasma for Atlantic salmon post-smolts exposed to carbon dioxide for 43 days (Reproduced from Fivelstad, 2012 with permission from Elsevier.)

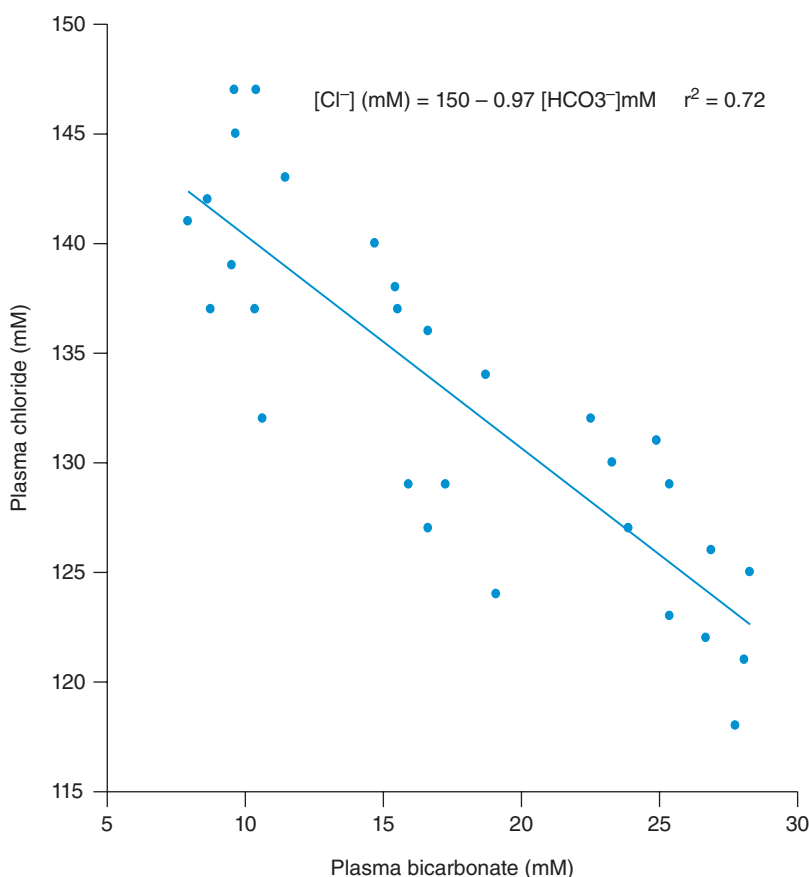


Fig. 10.5. The relationship between plasma bicarbonate and plasma chloride for Atlantic salmon post-smolts exposed to carbon dioxide. Individual measurements are given. (Reproduced from Fivelstad, 2012 with permission from Elsevier.)

cardiac and ventilator responses. Carbon dioxide receptors are important in avoidance reactions of fish subjected to levels above the thresholds. High carbon dioxide partial pressure may also interfere with the normal function of the central nervous system of fishes. According to Nilsson *et al.* (2012), high CO_2 in the water stimulates the GABA_A (γ -aminobutyric acid, type A) receptor in the central nervous system, resulting in malfunctioning of the senses as smell.

10.3.2 Physics and biology of dissolved gas supersaturation

In contrast to the previous sections where chemical parameters and gases were discussed in concentration units (mol/kg or mg/l), supersaturation depends on

pressure and therefore, this section will present information in terms of Torr or mm Hg. While these pressure terms are not SI units, they are more convenient for this subject than pressures expressed in Pa or kPa.

Supersaturation of individual gases

Fugacity is an idealized pressure that can be used to estimate the chemical potential of a real gas. At equilibrium, the fugacity of the gas phase is equal to the fugacity of the gas in the liquid phase:

$$f_i^g = f_i^l \quad (10.10)$$

where:

f_i^g = fugacity of *i*th gas in the gas phase (mm Hg)
 f_i^l = fugacity of *i*th gas in the liquid phase (mm Hg).

As the pressure approaches 1 atm, the fugacity of a real gas approaches its partial pressure. The partial pressure of gas is its pressure if it was the only gas in the volume:

$$P_i^g = \chi_i(BP - P_{uv}) \quad (10.11)$$

where:

P_i^g = partial pressure of i th gas (mm Hg)

BP = barometric pressure (mm Hg)

χ_i = mole fraction of the i th gas

P_{uv} = vapour pressure of water (mm Hg).

The pressure of an ideal gas in the liquid phase is termed 'gas tension' (Colt, 2012):

$$P_i^l = \left[\frac{C_i}{\beta_i} \right] A_i \quad (10.12)$$

where:

P_i^l = gas tension (mm Hg)

C_i = gas concentration (mg/l)

β_i = Bunsen coefficient of gas (l/l atm)

A = a constant that depends on the gas (mm Hg l/mg atm).

For an ideal gas, the partial pressure of a gas in the liquid phase is equal to its gas tension:

$$P_i^g = P_i^l \quad (10.13)$$

Of all the atmospheric gases, CO_2 is the least 'ideal' (Weiss, 1974). The implications of its non-ideal properties will be discussed in relation to gas supersaturation in the next section. The Bunsen coefficients, air solubility and partial pressures for atmospheric gases are presented in Table 10.1 for representative conditions. In terms of contribution to partial pressure, only nitrogen, oxygen, argon and carbon dioxide are significant. Of these four gases, carbon dioxide has the smallest contribution.

The partial pressures in Table 10.1 are based on the composition of gases in the atmosphere. Under anaerobic conditions, hydrogen and methane may be formed. The partial pressure of 1 mg/l of nitrogen, methane and hydrogen (Colt, 2012) for 20°C and 35 psu are 48.13 mm Hg, 38.01 mm Hg and 549.8 mm Hg, respectively. While methane and hydrogen could result in total gas supersaturation, this may not be biologically important because under anaerobic conditions, low DO levels and elevated hydrogen sulfide concentrations may be acutely lethal to most aquatic animals.

Depending on the magnitude of these two pressures, three conditions may occur:

Table 10.1. Bunsen coefficients, air solubility and partial pressures^a for atmospheric gases for temperature = 20°C and salinity = 35 psu. Further information is given in Colt (2012).

Gas	β_i (ml/(l atm))	C_o^+ (nmol/kg)	P_i^g (mm Hg)
Oxygen (O_2)	25.28	225,540	155.59
Nitrogen (N_2)	12.63	419,773	580.00
Argon (Ar)	27.84	11,075	6.94
Carbon dioxide (CO_2)	739.50	12,311	0.29
Helium (He)	7.46	2	3.89×10^{-3}
Neon (Ne)	8.83	7	1.35×10^{-2}
Krypton (Kr)	50.23	2	8.46×10^{-4}
Xenon (Xe)	86.51	0.3	6.69×10^{-5}
Hydrogen (H_2)	15.38	0.4	4.44×10^{-3}
Methane (CH_4)	27.87	2	1.79×10^{-3}
Nitrous oxide (N_2O)	532.80	7	1.16×10^{-4}

^a β_i , Bunsen coefficient of gas i ; C_o^+ , air solubility; P_i^g , partial pressure of i th gas.

$$P_i^l > P_i^g: \text{ supersaturated} \quad (10.14)$$

$$P_i^l = P_i^g: \text{ equilibrium} \quad (10.15)$$

$$P_i^l < P_i^g: \text{ undersaturated} \quad (10.16)$$

Total gas supersaturation

The sum of the partial pressures of all dissolved gases in the liquid and gas phases is equal to:

Liquid phase

$$\begin{aligned} \text{Total gas pressure} &= P_{\text{N}_2}^l + P_{\text{O}_2}^l + P_{\text{Ar}}^l \\ &\quad + P_{\text{CO}_2}^l + P_{uv} \end{aligned} \quad (10.17)$$

Gas phase

$$\begin{aligned} \text{Barometric pressure} &= P_{\text{N}_2}^g + P_{\text{O}_2}^g + P_{\text{Ar}}^g \\ &\quad + P_{\text{CO}_2}^g + P_{uv} \end{aligned} \quad (10.18)$$

The difference between the total gas pressure (TGP) and the local barometric pressure (BP) is called the ΔP :

$$\Delta P = \text{Total gas pressure} - BP \quad (10.19)$$

or

$$\text{Total gas pressure} = BP + \Delta P \quad (10.20)$$

TGP is the absolute pressure of the sum of the partial pressures + water vapour; ΔP is the gauge

pressure. TGP may also be expressed as a percentage of the local barometric pressure (BP):

$$\text{Total gas pressure (\%)} = \left[\frac{BP + \Delta P}{BP} \right] 100 \quad (10.21)$$

Similar to partial pressures, three conditions can occur for ΔP and TGP (%):

$$\Delta P > 0 \quad \text{or} \quad \text{TGP (\%)} > 100 \quad \text{supersaturated} \quad (10.22)$$

$$\Delta P = 0 \quad \text{or} \quad \text{TGP (\%)} = 100 \quad \text{equilibrium} \quad (10.23)$$

$$\Delta P < 0 \quad \text{or} \quad \text{TGP (\%)} < 100 \quad \text{undersaturated} \quad (10.24)$$

Biological impact of gas supersaturation

Elevated gas supersaturation levels can result in development of GBD, the most evident clinical sign is the development of bubbles on body surfaces and within the eyes and the gastrointestinal tract. Studies in hyperbaric physiology have shown that initial bubble formation depends on ΔP (D'Aoust and Clark, 1980). The ΔP value is the pressure that inflates bubbles. If $\Delta P \leq 0$, then bubbles cannot form regardless of the degree of supersaturation of a single gas.

The actual risk to an individual animal depends on the ΔP and the animal's position in the water column. The ΔP an animal experiences is equal to the difference between the total dissolved gas pressure and the local pressure (barometric + hydrostatic pressures). The uncompensated ΔP is equal to:

$$\Delta P_{\text{uncomp}} = \Delta P - \rho g Z \quad (10.25)$$

where:

ΔP = measured ΔP (mm Hg)

ρg = hydrostatic pressure of water (mm Hg/m of submergence)

Z = depth in water column (m).

The value of ρg depends slightly on both temperature and salinity. At 20°C and 0 psu salinity, the value of ρg is equal to 74.3 mm Hg/m.

General information on GBD is provided by D'Aoust and Clark (1980) and Weitkamp and Katz (1980). The following is a summary of the clinical signs of GBD (Colt, 2000) with emphasis on fish:

1. Subcutaneous emphysema (gas bubbles within skin tissues) is commonly on fins and tail, inside the mouth and operculum, and on the body surface.
2. Bubbles may be in the blood vessels, heart, kidney, spleen and liver. Long tubular bubbles may occur in the gill vessels.
3. Exophthalmia or 'popeye' results from the accumulation of gas in the eye.
4. The overinflation of swim bladders of small marine fish such as cod (*Gadus morhua* L.), sea bass (*Lates calcarifer*), striped bass (*Morone saxatilis*), lined rabbit fish (*Signanus lineatus*) and mullet (*Mugil cephalus*) is common in culture. The ability of small larval fish to regulate the volume of gas in the swim bladder may be limited.
5. The formation of bubbles in the gut of fish has been observed in herring (*Clupea harengus*), plaice (*Pleuronectes platessa*), channel catfish (*Ictalurus punctatus*), white sturgeon (*Acipenser transmontanus*) and striped bass. For small larval fish, formation of a single bubble may float them to the surface.
6. In salmonids, one of the more common clinical signs of exposure to gas supersaturation is the formation of bubbles in the scale pockets of the lateral line. The formation of bubbles in the lateral line results in reduced or eliminated ability of the fish to detect near-field water displacements and may decrease the fish's ability to avoid predation.

The first clinical sign of GBD is bubble formation in the gills and lateral line. The clinical signs are very dynamic; some clinical signs may rapidly disappear but this is not necessarily related to exposure levels or exposure time. Bubbles are lost most rapidly in gills, followed by the lateral line after sampling. Gill filaments from salmon smolts must be examined within 2 min after the gill arch is excised (Elston *et al.*, 1997).

The 4-day LC_{50} value (the lethal concentration required to kill 50% of the population in a specified time) of juvenile and adult fish ranges from 53 mm Hg to 230 mm Hg (Colt, 2000). The 30- to 35-day LC_{50} of fish ranges from 106 mm Hg to 117 mm Hg. The lethal tolerance of salmonids depends strongly on size or age. Salmon eggs are highly resistant to gas supersaturation because the pressure inside the egg ranges from 51 mm Hg to 76 mm Hg above local barometric pressure (Alderdice and Jensen, 1985). When aquatic animals are continuously exposed to ΔP s in the range of 20–100 mm Hg, a chronic type of GBD develops and is associated with extravascular symptoms such as bubble

formation in the gut and buccal cavity, hyperinflation or rupture of the swim bladder, and low-level mortality in juvenile animals over extended periods of time. In larval striped bass, clinical signs of GBD were observed at ΔP s as low as 22 mm Hg, and mortality was increased at 42 mm Hg (Cornacchia and Colt, 1984).

The water criterion for gas supersaturation established by the US Environmental Protection Agency (US EPA, 1976) is 110% of barometric pressure or a $\Delta P = 76$ mm Hg. This criterion is inadequate to protect the more sensitive species of non-salmonid fish or salmonids exposed to chronic gas supersaturation, particularly when water depth is limited. The Canadian Council of Ministers of the Environment (CCME, 1999) recommended a considerably lower criterion of $\Delta P = 24$ mm Hg. Colt (2000) suggested the following chronic water-quality criteria for gas supersaturation:

Very sensitive animals and experimental trials
 $\Delta P < 10$ mm Hg
Sensitive animals $\Delta P < 20$ mm Hg
Average animals under production conditions $\Delta P < 40$ mm Hg

The criterion suggested by Colt (2000) or CCME (1999) may be needed in culture systems but may be overly protective for environmental protection. It is important to keep in mind that ΔP s in excess of these criterion are commonly produced by natural processes and submergence of 14–55 cm below the water surface results in undersaturated conditions (Equation 10.25, $\Delta P < 0$ mm Hg).

Production of gas supersaturation

Physical, chemical and biological processes can produce gas supersaturation (Colt, 1986). Emphasis is placed on processes that occur in both natural and culture conditions.

HEATING. The solubility of atmospheric gases decreases with increasing temperature. If the system is closed (no gas transfer), the partial pressure of the dissolved gases will increase when heated. This type of heating can occur in a pressurized water heater, with groundwater or springs, and in open ponds in the spring and summer. All three examples can produce acutely lethal dissolved gas levels.

BUBBLE ENTRAINMENT. When bubbles are carried down into the water column or when gas and water are present together at elevated pressures, gas

supersaturation may be produced. This commonly occurs from leaks on the suction side of the pumps, clogging of intake structures so that flowing water does not completely fill the pipe, or an intake pipe that is not completely submerged. Air entrainment is more serious in seawater because of smaller bubbles that result in a significant increase in the overall gas transfer rate (Kils, 1977). Another common example of air entrainment occurs below high dams or waterfalls. In the ocean, breaking waves carry air bubbles down into the water column and a significant fraction of the gas may dissolve.

PHOTOSYNTHESIS. This process consumes CO_2 and produces oxygen which has a much higher partial pressure per unit mass. However, it is unlikely that photosynthesis by itself can produce GBD unless the total gas pressure is supersaturated. The reported observation of GBD from photosynthesis did not measure gas supersaturation, only DO (Weitkamp and Katz, 1980). Under conditions of intense photosynthesis, solar heating can result in significant increases in water temperature. Lethal levels of gas supersaturation can result from the impact of heating on nitrogen, argon and oxygen as well as from the contribution from the added oxygen produced from photosynthesis. Due to the planktonic activity rhythm, DO in the surface layer often fluctuates between saturation deficit at night/morning and supersaturation in the afternoon, and during blooms the DO amplitudes are maximized (Fig. 10.6).

PHYSIOLOGICAL PROCESSES. While typically bubbles cannot form when $\Delta P \leq 0$, it may be possible to form bubbles inside an animal when the ΔP in the water is less than zero (Colt, 1986). The condition for bubble formation ($\Delta P \geq 0$) should be applied inside the animal where bubbles form and not to the ambient water. Some fish have the ability to generate very high partial pressures in the swim bladder and eyes even when the dissolved gas concentrations in the surrounding water are close to equilibrium (Wittenberg and Wittenberg, 1974; Fänge, 1983). These processes may be more important in culture systems because of limited depth for hydrostatic compensation.

Impact of climate change on gas supersaturation – calculation for future conditions

The accuracy of our assessment of the potential impact of climate change on gas supersaturation depends on how well we can predict the future.

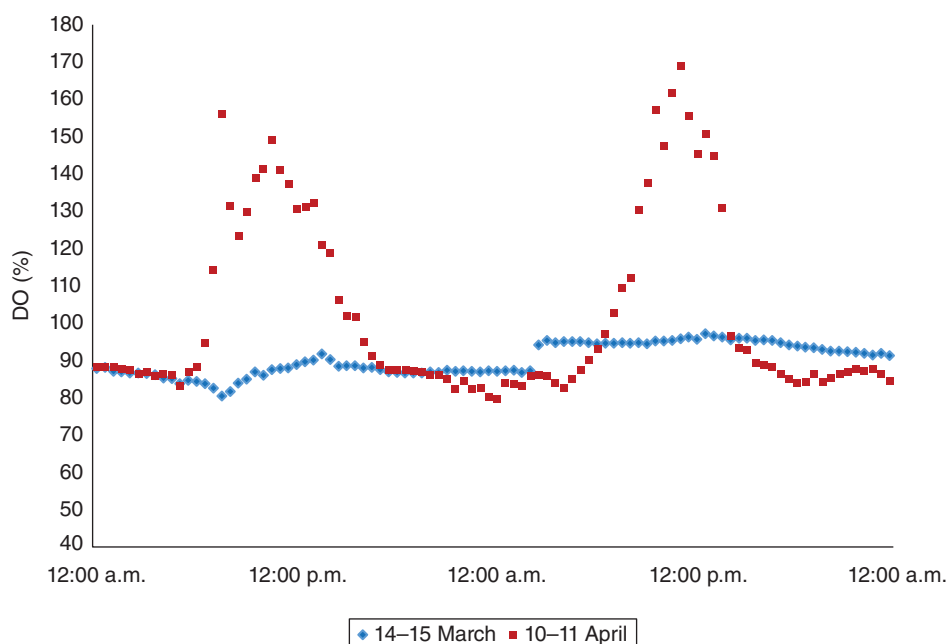


Fig. 10.6. Dissolved oxygen (DO) concentration at 3 m depth in a fjord, south-west Norway during 2 day periods in March and April 2011. Temperature: 3.0–3.5°C (March), 3.5–4.0°C (April). The ‘spring algal bloom’ created high diurnal DO fluctuation during the first half of April. (From Bergheim *et al.*, 2011, with permission to reproduce given by A. Bergheim, International Research Institute of Stavanger (IRIS), Norway.)

This section will explore the potential impact of: (i) increasing carbon dioxide levels in the atmosphere; (ii) changes in water temperature; and (iii) changes in DO levels. The time frame for this analysis is based on 100 years (2000–2100). While 2100 is a long time in the future, it is important to realize that some of the changes that have occurred operate on timescales that are measured in decades to centuries. This is especially true for marine waters that have been loaded with CO₂ and have been carried down into the ocean depths. Some of these waters will not be carried back to the surface for 1000 years or more; nothing we do in the near term on the surface will have any impact on biological and chemical processes occurring in these waters.

IMPACT OF INCREASING CO₂ LEVELS IN THE ATMOSPHERE ON GAS SUPERSATURATION. In the *Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (IPCC, 2013a) a series of atmospheric CO₂ predictions were developed and denoted as representative concentration pathways (RCPs). The RCPs are identified by their approximate total radiative forcing in year 2100 relative to 1750:

RCP2.6	2.6 W/m ²
RCP4.5	4.5 W/m ²
RCP6.0	6.0 W/m ²
RCP8.5	8.5 W/m ²

These four RCPs include one mitigation scenario leading to a very low forcing level (RCP2.6), two stabilization scenarios (RCP4.5 and RCP6.0), and one scenario with very high greenhouse gas emissions (RCP8.5). Each RCP provides spatially resolved data sets of land use change and sector-based emissions of air pollutants, and it specifies annual greenhouse gas concentrations and anthropogenic emissions up to 2100. RCPs are based on a combination of integrate assessment models, simple climate models, atmospheric chemistry and global carbon cycle models (IPCC, 2013a).

The concentrations of CO₂ for the RCPs are presented in Table 10.2 (IPCC, 2013b). The four RCPs assume that the concentration of atmospheric CO₂ was 368.9 ppm in the year 2000. The year 2100 values range from 420.9 ppm for RCP2.6 to 935.9 ppm for RCP8.5.

For ideal gases, the partial pressure and gas tension of an ideal gas can be computed from

Table 10.2. Concentration of carbon dioxide for four representative concentration pathways (RCPs) presented in Table AII.4.1 (IPCC, 2013b).^a

Year	Carbon dioxide (ppm)			
	RCP2.6	RCP4.5	RCP6.0	RCP8.5
2000	368.9	368.9	368.9	368.9
2005	378.8	378.8	378.8	378.8
2010	389.3	389.1	389.1	389.3
2020	412.1	411.1	409.4	415.8
2030	430.8	435.0	428.9	448.8
2040	440.2	460.8	450.7	489.4
2050	442.7	486.5	477.7	540.5
2060	441.7	508.9	510.6	603.5
2070	437.5	524.3	549.8	677.1
2080	431.6	531.1	594.3	758.2
2090	426.0	533.7	635.6	844.8
2100	420.9	538.4	669.7	935.9

^aPermission to reproduce the table has been given by the IPCC secretariat, Geneva, Switzerland.

Equations 10.11 and 10.12. This requires knowledge of the concentration of gas in solution, mole fraction, barometric pressure and water temperature. These equations do not assume that the partial pressure and gas tensions are at equilibrium ($P_i^l = P_i^g$). These two equations are useful when one knows the concentration of a gas in solution and need to compute partial pressures or component gas values.

For assessing the impact of CO₂ increases, we need to estimate the partial pressure in equilibrium with a predicted atmospheric concentration such as listed in Table 10.2. One approach would be to compute future concentrations in mg/l or micro-mole/kg using a carbonate model and convert these to mm Hg using Equation 10.12.

‘CO₂ is many times more soluble than any of the gases treated previously, and in the gas phase its departures from the ideal gas approximation are large compared to the accuracy with which its solubility can be measured’ (Weiss, 1974). For this gas, the partial pressure should be replaced by its fugacity (Weiss, 1974):

$$f_{\text{CO}_2} = \chi_{\text{CO}_2} (BP - P_{wv}) \exp \left[\frac{BP(B + 2\delta)}{RT} \right] \quad (10.26)$$

where:

f_{CO_2} = fugacity (atm)

B = second virial coefficient for the gas (l/mol)

δ = cross-virial coefficient for carbon dioxide and air (l/mol)

R = universal gas constant 0.8205601 (l atm/mol°K)

T = absolute temperature (°K)

Note that the first part of Equation 10.26 is identical to Equation 10.11. The $\exp \left[\frac{BP(B + 2\delta)}{RT} \right]$ term is a correction for the non-ideal properties of carbon dioxide gas.

For CO₂ levels in RCP8.5, its fugacity is presented for fresh water, seawater and brackish water at two temperatures (Table 10.3). For the years 2000–2100, the fugacity of CO₂ varies from 0.277 mm Hg to 0.703 mm Hg for 4°C and from 0.268 mm Hg to 0.680 mm Hg for 30°C. Salinity has little impact on the fugacity and its only impact in Equation 10.26 is on the P_{wv} term. While the fugacity increases by a factor of 2.54 over the 100 years, its absolute value is small compared with typical gas supersaturation values (for a $\Delta P = 100$ mm Hg, the total gas pressure = 760 + 100 = 860 mm Hg). Increases in atmospheric CO₂ will not increase the risk from GBD.

At the bottom of Table 10.3, the absolute difference and percentage difference between the fugacity and partial pressure is presented. While the non-ideal properties of CO₂ are important for carbonate chemistry, Equation 10.12 is accurate enough for routine gas supersaturation work.

IMPACT OF CHANGES IN WATER TEMPERATURE ON GAS SUPERSATURATION.

The potential impact of temperature changes on gas supersaturation is much more complex than the impact of changes in atmospheric CO₂. The impact of climate change on water temperature depends strongly on location and the rate of temperature change may be as important as the total change. IPCC (2013b) presents global mean surface temperature changes for the four RCPs for 5–95 percentiles and are summarized in Table 10.4 for 50–95 percentiles. Additional information is presented for specific locations in graphical format (IPCC, 2013c).

Assuming no transfer of gases, the resulting ΔP for O₂, N₂ + Ar, and CO₂ gases can be computed from (Colt, 2012):

$$\begin{aligned} \Delta P = & Sat_{\text{O}_2} \frac{C_{\text{O}_2, T_i}^*}{\beta_{\text{O}_2, T_f}} A_{\text{O}_2} \\ & + Sat_{\text{N}_2 + \text{Ar}} \frac{C_{\text{N}_2 + \text{Ar}, T_i}^*}{\beta_{\text{N}_2 + \text{Ar}, T_f}} A_{\text{N}_2 + \text{Ar}} \\ & + Sat_{\text{CO}_2} \frac{C_{\text{CO}_2, T_i}^*}{\beta_{\text{CO}_2, T_f}} A_{\text{CO}_2} + P_{wv, T_f} - BP \end{aligned} \quad (10.27)$$

Table 10.3. Fugacity of carbon dioxide (in mm Hg) as a function of mole fraction, temperature and salinity. (Based on carbon dioxide concentrations in RCP8.5 (Table 10.2), Weiss, 1974, and Ambrose and Lawrenson, 1972.)

CO ₂ (ppm)	Fresh water		Seawater (35 psu)		Brackish water (10 psu)	
	4°C	30°C	4°C	30°C	4°C	30°C
368.9	0.277	0.268	0.277	0.268	0.277	0.268
378.8	0.284	0.275	0.284	0.275	0.284	0.275
389.3	0.292	0.283	0.292	0.283	0.292	0.283
415.8	0.312	0.302	0.312	0.302	0.312	0.302
448.8	0.337	0.326	0.337	0.326	0.337	0.326
489.4	0.367	0.355	0.367	0.356	0.367	0.355
540.5	0.406	0.392	0.406	0.393	0.406	0.393
603.5	0.453	0.438	0.453	0.439	0.453	0.438
677.1	0.508	0.492	0.508	0.492	0.508	0.492
758.2	0.569	0.551	0.569	0.551	0.569	0.551
844.8	0.634	0.613	0.634	0.614	0.634	0.614
935.9	0.703	0.680	0.703	0.680	0.703	0.680
Difference ^a	0.00292	0.00196	0.00292	0.00196	0.00292	0.00196
Percentage difference ^b	0.41%	0.29%	0.41%	0.29%	0.41%	0.29%

^a'Difference' is the maximum difference between the fugacity and the partial pressure of CO₂ in mm Hg.

^b'Percentage difference' is the percentage difference of the two pressures (i.e. the fugacity and the partial pressure of CO₂).

where:

Sat_i = saturation for gas i (dimensionless)

C_{i,T_i}^* = saturation concentration for gas i , initial temperature (mg/l)

β_{i,T_f} = Bunsen coefficient for gas i , final temperature (l/l atm)

A_i = a constant for gas i (Colt, 2012)

P_{wv,T_f} = vapour pressure of water, final temperature (mm Hg)

BP = barometric pressure (mm Hg)

T_i = initial temperature (°C)

T_f = final temperature (°C).

For saturated water, Equation 10.27 reduces to:

$$\Delta P = \frac{C_{O_2,T_i}^*}{\beta_{O_2,T_f}} A_{O_2} + \frac{C_{N_2+Ar,T_i}^*}{\beta_{N_2+Ar,T_f}} A_{N_2+Ar} + \frac{C_{CO_2,T_i}^*}{\beta_{CO_2,T_f}} A_{CO_2} + P_{wv,T_f} - BP \quad (10.28)$$

This equation is simpler to solve, as it does not require information on component gas concentrations. Implicit in both Equations 10.27 and 10.28 is the assumption that concentrations of all gases on a mg/l basis is constant (no gas transfer across the air-water boundary and no chemical reactions involving oxygen or CO₂. Changes in DO will be discussed in the following section. These assumptions may be more valid for temperature changes occurring over weeks to months than over years to decades.

The temperature increase for the RCP8.5 (50 percentile) is given in Table 10.4. The resulting ΔP values for saturated water (Equation 10.28) are presented in Table 10.5.

Similar results are presented in Table 10.6 for an initial ΔP = 160 mm Hg (DO = 8.00 mg/l, CO₂ = 0.80 mg/l, CO₂ (atm) = 400 ppm and BP = 760 mm Hg).

Note for a constant ΔP and DO, the saturation of the gases (as a decimal fraction) used in Equation 10.27 depends on both temperature and salinity and are presented in Table 10.7 for six temperature/salinity combinations used in Table 10.6.

Heating has a much larger impact on colder initial temperatures. For fresh water, the resulting ΔP was 67.4 mm Hg for 4°C and 39.8 mm Hg for 30°C. Salinity has a minor impact on the resulting ΔP . The impact of heating is larger for waters initially supersaturated (Table 10.6). The individual gases do not contribute to the resulting ΔP equally. For example, for T_i = 30°C, S = 10 psu and ΔT 3.57°C, the total change in ΔP = 45.78 mm Hg, but the individual contributions (mm Hg) were equal to:

ΔP_{O_2}	8.63
ΔP_{N_2}	29.64
ΔP_{Ar}	0.42
ΔP_{wv}	7.09
Total	45.78

Table 10.4. Global temperature increases for four representative concentration pathways (RCPs) and two percentiles (Table AII.7.5, IPCC, 2013b).^a

Years	RCP2.6		RCP4.5		RCP6.0		RCP8.5	
	50%	95%	50%	95%	50%	95%	50%	95%
1850–1990	−0.61		−0.61		−0.61		−0.61	
1986–2005	0.00		0.00		0.00		0.00	
2010	0.36	0.62	0.36	0.59	0.36	0.64	0.37	0.62
2020	0.55	1.07	0.59	0.83	0.55	0.90	0.66	0.99
2030	0.74	1.24	0.82	1.22	0.74	1.17	0.94	1.39
2040	0.88	1.50	1.04	1.57	0.95	1.41	1.29	1.77
2050	0.94	1.65	1.24	1.97	1.15	1.81	1.70	2.37
2060	0.93	1.71	1.44	2.19	1.32	2.18	2.16	2.99
2070	0.89	1.71	1.54	2.32	1.58	2.52	2.60	3.61
2080	0.94	1.79	1.62	2.54	1.81	2.88	3.05	4.22
2090	0.94	1.79	1.68	2.59	2.03	3.24	3.57	4.81

^aPermission to reproduce the table has been given by the IPCC secretariat, Geneva, Switzerland.

Table 10.5. Impact of heating on ΔP (initial gas level saturated, mm Hg, temperature rise equal to RCP8.5, 50 percentile).^a (Based on Table 10.4, equations in the present chapter and Colt, 2012.)

Δt (°C) ^b	Fresh water		Seawater (35 psu)		Brackish water (10 psu)	
	4°C	30°C	4°C	30°C	4°C	30°C
0.00	0.0	0.0	0.0	0.0	0.0	0.0
0.37	7.0	4.2	6.3	3.9	6.8	4.1
0.66	12.4	7.4	11.3	6.9	12.1	7.3
0.94	17.7	10.6	16.1	9.8	17.2	10.3
1.29	24.3	14.5	22.0	13.5	23.6	14.2
1.70	32.0	19.0	29.0	17.7	31.2	18.7
2.16	40.7	24.2	36.9	22.5	39.6	23.7
2.60	49.0	29.0	44.4	27.0	47.7	28.5
3.05	57.5	34.0	52.1	31.7	56.0	33.4
3.57	67.4	39.8	61.0	37.0	65.5	39.0

^a Δp , Total gas pressure expressed as a gauge pressure (see Equation 10.19 in text).

^b Δt , The rise in temperature.

Table 10.7. Saturation of the gases (as a decimal fraction) in relation to six temperature/salinity combinations used in Table 10.6.

T_i (°C)	Salinity	Sat_{N_2+Ar}	Sat_{O_2}	Sat_{CO_2}
4	0	1.3720	0.6103	0.7254
30	0	1.2622	1.0584	1.6742
4	35	1.3288	0.7727	0.8680
30	35	1.2024	1.2828	1.9399
4	10	1.3607	0.6528	0.7634
30	10	1.2463	1.1180	1.7461

Table 10.6. Impact of heating on ΔP (initial ΔP = 160 mm Hg, temperature rise equal to RCP8.5, 50 percentile). ^a (Based on Table 10.4 and equations in the present chapter and Colt, 2012.)

Δt (°C) ^b	Fresh water		Seawater (35 psu)		Brackish water (10 psu)	
	4°C	30°C	4°C	30°C	4°C	30°C
0.00	160.0	160.0	160.0	160.0	160.0	160.0
0.37	168.3	164.9	167.5	164.6	168.1	164.8
0.66	174.8	168.7	173.5	168.2	174.5	168.5
0.94	181.2	172.4	179.2	171.6	180.6	172.2
1.29	189.0	177.0	186.4	175.9	188.3	176.7
1.70	198.3	182.4	194.8	180.9	197.3	181.9
2.16	208.7	188.4	204.2	186.5	207.4	187.8
2.60	218.6	194.1	213.2	191.9	217.1	193.5
3.05	228.8	200.0	222.4	197.4	227.0	199.2
3.57	240.6	206.7	233.0	203.7	238.4	205.8

^a Δp , Total gas pressure expressed as a gauge pressure (see Equation 10.19 in text).

^b Δt , The rise in temperature.

Nitrogen contributes more to the impact of heating because of its larger atmospheric concentration (Table 10.1) and lower solubility (higher partial pressure per mg/l).

Typical limnology and biological analysis emphasize the impact of temperature on the saturation concentration of DO. The impact of temperature on nitrogen and argon gases receives less attention because these gases are largely biologically inert. While the saturation concentration of oxygen does significantly decrease with

increasing temperature (Fig. 10.7), its partial pressure changes little with temperature (Fig. 10.8). In nature, the 100% oxygen saturation is seldom found; the saturation is often higher or lower than this.

IMPACT OF CHANGES IN DO LEVELS ON GAS SUPERSATURATION. Once supersaturation has

occurred it can be stable for months or years. Hydrostatic pressure has a significant impact on the ΔP_{uncomp} (Equation 10.25). For example, for $\Delta P = 120$ mm Hg and $\rho g = 74.3$ mm Hg/m, the $\Delta P_{uncomp} = -28.6$ mm Hg. Therefore, below this depth there is no tendency for bubbles to form because the water is undersaturated with regard to total gas pressure. In addition, bubble formation requires

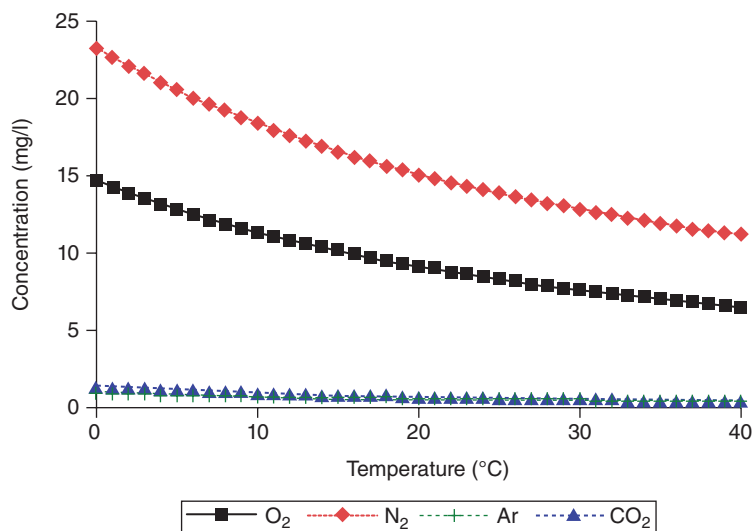


Fig. 10.7. Saturation concentration of major atmospheric gases in mg/l (fresh water, barometric pressure = 760 mm Hg, CO₂ (atm) = 400 ppm). (Based on Colt, 2012.)

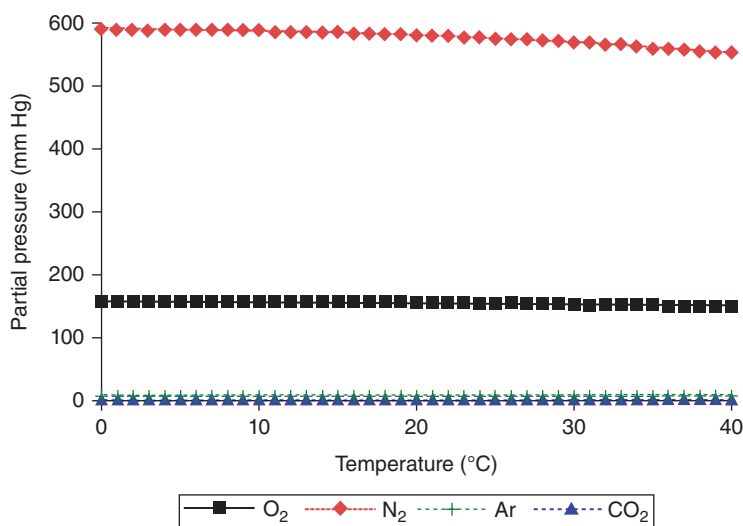
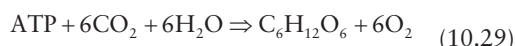


Fig. 10.8. Partial pressure of major atmospheric gases (in mm Hg) as temperature changes from 0°C to 40°C (computed from Equation 10.11 and CO₂ (atm) = 400 ppm).

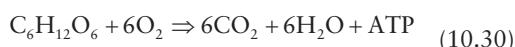
nucleation sites and high levels of supersaturation may not result in bubble formation. Simple gas transfer can only occur at the air–water interface.

As a result, biological processes such as photosynthesis or respiration may be responsible for significant changes in dissolved gas concentrations and partial pressures. Two of the most important biological processes are:

Photosynthesis:



Respiration:



Conversion between mg/l and mm Hg are presented for the major atmospheric gases in Table 10.8.

At 20°C in fresh water, the production of 1 mg/l of oxygen will increase the total gas pressure by 16.5 mm Hg/l:

1.38 mg of carbon dioxide is needed to produce 1 mg of oxygen
 For oxygen at 20°C, 17.106 mm Hg/(mg/l) Table 10.8, column 2
 For carbon dioxide at 20°C, 0.44168 mm Hg/(mg/l) Table 10.8, column 5
 $1 \text{ mg/l} \times 17.106 \text{ mm Hg} = 17.106 \text{ mm Hg}$
 $-1.38 \text{ mg/l} \times 0.44168 \text{ mm Hg} = -0.610 \text{ mm Hg}$
 $+16.496 \text{ mm Hg}$

At 40°C, the total gas pressure is increased by 23.0 mm Hg/(mg/l).

Photosynthesis can significantly increase the partial pressure of oxygen as well as total gas pressure. Respiration has the opposite impact.

Because of hydrostatic compensation, the impact of photosynthesis and respiration is more important in the upper 1–5 m of the water column. These processes have important biological impacts below these levels, but not on gas supersaturation or GBD.

The general effects of climate change on the freshwater system will probably increase water temperatures and decrease DO (Ficke *et al.*, 2007). The changes in DO will depend on a variety of factors such as ambient temperatures, latitude, cloud cover, wind mixing, soluble and particulate compounds, and the composition of the local biological communities. The impact of climate change on DO may be quite different in lakes and streams. Blumberg and DiToro (1990) estimated that 1 mg/l of DO could be lost from the upper layer of Lake Erie. Bello *et al.* (2017) estimated the DO could decrease by 0.39–0.53 mg/l depending on location

in a large tropical stream in Malaysia. Matear and Hirst (2003) modelled the decrease in DO at 2100 as a function of depth and latitude:

At the equator:	0–0.33 mg/l
40°S to 40°N (except at the equator)	0.33–0.66 mg/l
Above 40°N and below 40°S	0.99–1.32 mg/l

Assuming representative temperature for these decreases and partial pressure conversions in Table 10.8, it is reasonable to expect a 10–20 mm Hg decrease in the partial pressure of oxygen and the total gas pressure.

The combined impact of temperature increases and DO decreases may depend strongly on the magnitude of each process on a seasonal and decadal time frame. This analysis has assumed the simplest case: both occur at the same time. Much more detailed modeling is needed to explore the potential interaction of climate change on gas supersaturation in a wide variety of marine and freshwater conditions.

SUMMARY OF IMPACTS. Because of the high solubility of CO₂, increases in the atmospheric level of the gas will have a negligible impact on gas supersaturation or GBD. This is not the case for climate change heating. For waters initially saturated, RCP8.5, 50 percentile temperature increases, the resulting ΔP s are in the range of 40–67 mm Hg at year 2100. Estimated reductions in DO could reduce this ΔP by 10–20 mm Hg. For sensitive species, climate change impacts on gas supersaturation may occur in the 2060–2090 time period. More serious impacts could be expected for the 83 or 95 percentile temperature increases for RCP8.5.

Recent findings on GBD

LETHAL TOTAL GAS LEVELS. Many of the recent publications on lethal total gas levels are based on problems in China resulting from the construction of a number of high dams. Because of the very high total gas pressure produced, climate change will have little impact on the lethal impacts but could increase chronic problems downstream of the dams. This section will focus on new observations on bubble formation and development of GBD at ΔP s in the range of –15–40 mm Hg.

BUBBLE FORMATION IN THE SWIM BLADDER AND GAS-INTESTINAL TRACT. Palma *et al.* (2014) found the long snout seahorse (*Hippocampus guttulatus*) fed with DAHA-Selco-enriched *Artemia* developed

Table 10.8. Partial pressure (mm Hg/mg/l) as a function of temperature and salinity. (Based on equations in the present chapter and in Colt, 2012.)

Temperature (°C)	Fresh water				Seawater (35 psu)			
	O ₂	N ₂	Ar	CO ₂	O ₂	N ₂	Ar	CO ₂
0	10.822	25.359	7.921	0.22260	13.827	32.929	10.095	0.26713
1	11.126	26.022	8.141	0.23154	14.182	33.707	10.352	0.27769
2	11.431	26.688	8.362	0.24071	14.539	34.487	10.610	0.28849
3	11.739	27.355	8.585	0.25009	14.897	35.266	10.869	0.29952
4	12.048	28.024	8.809	0.25969	15.257	36.046	11.129	0.31079
5	12.359	28.695	9.034	0.26951	15.618	36.824	11.390	0.32229
6	12.671	29.366	9.260	0.27954	15.979	37.602	11.651	0.33403
7	12.985	30.037	9.488	0.28979	16.342	38.378	11.913	0.34598
8	13.300	30.708	9.715	0.30026	16.705	39.152	12.175	0.35816
9	13.616	31.378	9.944	0.31093	17.068	39.924	12.438	0.37056
10	13.933	32.047	10.173	0.32182	17.432	40.693	12.700	0.38317
11	14.250	32.714	10.403	0.33291	17.795	41.458	12.962	0.39599
12	14.568	33.379	10.633	0.34421	18.159	42.220	13.225	0.40902
13	14.886	34.042	10.863	0.35572	18.522	42.978	13.487	0.42224
14	15.204	34.702	11.093	0.36742	18.884	43.731	13.748	0.43566
15	15.522	35.359	11.323	0.37932	19.246	44.478	14.009	0.44927
16	15.840	36.011	11.553	0.39142	19.607	45.221	14.270	0.46306
17	16.157	36.660	11.782	0.40371	19.967	45.958	14.529	0.47703
18	16.474	37.303	12.011	0.41618	20.326	46.688	14.788	0.49116
19	16.790	37.942	12.240	0.42884	20.683	47.413	15.046	0.50546
20	17.106	38.575	12.468	0.44168	21.039	48.130	15.303	0.51992
21	17.420	39.203	12.695	0.45470	21.393	48.840	15.558	0.53452
22	17.733	39.824	12.921	0.46788	21.745	49.542	15.812	0.54927
23	18.045	40.438	13.146	0.48123	22.096	50.237	16.065	0.56415
24	18.356	41.045	13.369	0.49475	22.444	50.923	16.317	0.57916
25	18.664	41.645	13.592	0.50842	22.790	51.601	16.566	0.59428
26	18.971	42.237	13.813	0.52224	23.133	52.269	16.815	0.60952
27	19.277	42.820	14.033	0.53621	23.474	52.929	17.061	0.62485
28	19.580	43.395	14.251	0.55032	23.812	53.579	17.305	0.64028
29	19.881	43.961	14.468	0.56457	24.148	54.219	17.548	0.65580
30	20.180	44.518	14.682	0.57895	24.480	54.848	17.788	0.67139
31	20.477	45.065	14.895	0.59345	24.810	55.467	18.026	0.68704
32	20.771	45.601	15.105	0.60807	25.136	56.076	18.262	0.70275
33	21.062	46.128	15.313	0.62280	25.459	56.673	18.495	0.71851
34	21.351	46.643	15.519	0.63764	25.779	57.259	18.726	0.73431
35	21.637	47.147	15.723	0.65258	26.095	57.832	18.954	0.75014
36	21.920	47.639	15.924	0.66761	26.407	58.394	19.180	0.76599
37	22.200	48.119	16.122	0.68274	26.716	58.943	19.402	0.78185
38	22.477	48.587	16.318	0.69794	27.022	59.479	19.622	0.79771
39	22.750	49.042	16.510	0.71321	27.323	60.002	19.839	0.81357
40	23.021	49.483	16.699	0.72855	27.620	60.512	20.052	0.82940

a severe condition of overinflation of the gas bladder and that after 48 h forced air into the gut. High mortality occurred within 120 h after the first meal. When fed natural copepods, the seahorse juveniles continued normal feeding with no swim bladder or gastrointestinal problems. They concluded that the overinflation of the swim bladder was due

to a nutritionally unbalanced diet but presented no mechanism how this resulted in swim-bladder overinflation.

EYE PROBLEMS. White sea bass (*Atractoscion nobilis*) have suffered from exophthalmia (popeye) and intraocular emphysema (accumulation of air

in the eyeball) in both hatchery and net-pen systems (Smiley *et al.*, 2011). Cutaneous emphysemas on the fins, roof of the mouth and body surfaces developed at relatively high ΔP s (121 mm Hg and above). The prevalence and severity of corneal emphysemas increased sharply with increasing ΔP s (Smiley *et al.*, 2011). The prevalence was larger at 23°C compared with 18°C. For large fish and 23°C, intraocular emphysema were observed at -15 mm Hg and above and for small fish and 23°C at +15 mm Hg. Smiley *et al.* (2012) showed the incidence of eye damage at $\Delta P = -15$ mm Hg ranged from 10% to 90% of the fish examined. A significant dose-response relationship between ΔP and clinical damage to the eye was only found in two out of 16 cases. It is likely that the observed clinical signs are the result of high partial pressures produced by the choroid rete mirabile of the eye and inadequate hydrostatic pressure to prevent bubble development.

CHRONIC EXPOSURE. Gunnarsli *et al.* (2008) found gas supersaturation had no impact on the swimming performance, growth and mortality of juvenile cod (30–55 g) up to $\Delta P = 38$ mm Hg over a 7-week study. A low level of exophthalmia occurred at 22.4 mm Hg and 37.4 mm Hg. A water quality criterion of less than 22.4 mm Hg was suggested.

Short-term exposure (16 days) of rainbow trout to $\Delta P = 23$ mm Hg did not affect feed intake, behaviour or result in clinical signs of GBD (Skov *et al.*, 2013). It did significantly reduce apparent lipid digestibility, feed conversion and the thermal growth coefficient. There was a significant decrease in available metabolizable energy. Over the entire 25-day experiment, there were no significant differences in production variable suggesting that rainbow trout could adapt to moderate levels of gas supersaturation.

10.4 Controls and Preventions

In the future, fish will be exposed to elevated CO_2 concentration (and partial pressure) in blood plasma, compared with today and there will be a global change in fish body composition. The general response to an elevated bicarbonate concentration is reduced plasma chloride as a result of branchial HCO_3^- uptake (Esbaugh *et al.*, 2012) and chloride extrusion. According to Ishimatsu *et al.* (2005), Cl^- decreases with a nearly equimolar increase in HCO_3^- in marine teleost fish under low levels of hypercapnia

(8–16 mm Hg). Fivelstad *et al.* (2017) also found a linear decrease in plasma chloride in Atlantic salmon post-smolts exposed to 2–14 mm Hg CO_2 . How the increased CO_2 concentration, increased bicarbonate concentration and decreased plasma chloride in teleost fish will affect fish anatomy and fish physiology is only partly known (Esbaugh *et al.*, 2005). However, fish can live under such conditions in aquaculture for a long time.

There is limited knowledge about how fish will adapt to impacts of climate change. According to Merilä and Hoffmann (2016) there are three different mechanisms:

1. Changing the genetic constitution.
2. Phenotypic plasticity which means that the genotypes have the ability to express different phenotypes (change in breeding schedule, physiology).
3. Migration to a location having better environmental conditions.

Miller *et al.* (2012) found that non-genetic parental effects can increase CO_2 tolerance in fish. More specifically, they studied how the CO_2 tolerance of anemonefish (*Amphiprion melanopus*) are influenced by the CO_2 exposure of their parents and found that growth of juveniles exposed to increased CO_2 are strongly dependent on the CO_2 exposure of their parents. This is an example of phenotypic plasticity.

However, Welch *et al.* (2014) showed that olfactory preferences were influenced by elevated CO_2 and found limited possibilities for within-generation acclimatization and the effects were undiminished by transgenerational acclimatization.

According to Ishimatsu *et al.* (2005), CO_2 tolerance varies among different fish species and also among development stages within a species. Thus tolerant species may be favoured by the CO_2 -induced seawater acidification. However, the increased CO_2 levels are low compared with those under aquaculture conditions. Generally few or no effects are found on salmonids at concentrations below 6000 μatm . It should, be noted that some of the parameters measured for aquaculture research differ from those in CO_2 acidification research, as the focus in the latter also include olfactory preferences and brain function (Welch *et al.*, 2014).

In freshwater studies, there are significant variations in acidic water tolerance among salmonid species, and large genetic variation among strains has been documented (Gjedrem and Rosseland, 2012). However, the acidification of streams during

1960–1980 in southern Norway occurred too fast for the fish to adapt by natural selection. Andren *et al.* (1989), in a study of moor frogs (*Rana alvaris*), found that adaptation to a new environment takes 15 generations. The CO₂-induced acidification of seawater is a much slower process, compared with acidification of fresh water from acid rain, and therefore adaptation is possible.

Cramer and McIntyre (1975) investigated the tolerance to supersaturation in several stocks of Chinook salmon from Oregon coastal streams and from the Columbia River. The most tolerant stocks were those stocks with the longest exposure history and the study indicated that tolerance was inherited.

10.5 Conclusion and Suggestions for Future Studies

The main change in pH may occur between pH 7.5 and 5.5 in fresh water, depending on alkalinity and prior exposure to acid rain. In seawater alkalinity is rather stable, mainly in the range of 2.0–2.4 mM and the reduction in pH is smaller. The present day pH in the ocean is about 8.0, and upwelling seawater may be down to pH 7.5, while projected future upwelling seawater may be down to pH 7.1. Simulation models have indicated the reduction in pH to be 0.7–0.8 pH units by the year 2300 (Caldeira and Wickett, 2003).

Nephrocalcinosis has been found in carbon dioxide-exposed salmonids in soft fresh water, hard fresh water and in seawater. This research has been performed to find safe levels for carbon dioxide under aquaculture conditions, and the carbon dioxide levels have, however, been above about 6000 μ atm, conditions that at present are not considered realistic for carbon dioxide acidification of the global fresh and seawater. Future research on nephrocalcinosis should especially consider carbon dioxide levels between 400 μ atm and 6000 μ atm for different fish species and life stages.

10.5.1 Hypercapnia in soft fresh water

In soft fresh water CO₂ reduces water pH and the changes in Al chemistry may be detrimental to Atlantic salmon smolts, even at low Al concentrations. Although pH as a single factor may not have toxic effects, it may have minor effects when combined with moderate changes in CO₂ levels. Most soft water also contains Al, and

metal toxicity may thus be important in CO₂-induced soft water acidification.

10.5.2 Hypercapnia in hard fresh water

In hard water dose-dependent reduction in growth, yolk-to-tissue conversion and maximal oxygen uptake capacity has been found in pink salmon. Effects on olfactory responses and behaviour also seemed to be dose dependent.

CO₂ receptors are important in avoidance reactions of fish when levels are above thresholds. High partial pressure of the gas may also interfere with the normal function of the central nervous system. This means that the central nervous system and the peripheral nervous system, including senses, are of great importance in CO₂-induced water acidification. The total water quality has to be studied carefully in such experiments to avoid influence from metal toxicity and the concentrations of metals (e.g. Al) in the gills should be examined.

10.5.3 Hypercapnia in seawater

A negative linear relationship between pCO₂ (range 0.6–11.6 mm Hg; about 790–15,790 μ atm) and specific growth rate was observed for post-smolts (34‰ seawater at 10°C) in 12 days (Fivelstad *et al.*, 2017). Stress related to weighing and blood sampling may have added to the effect of CO₂, indicating that fish exposed to increased pCO₂ are more susceptible to other factors and vice versa. This means that sampling periods may influence the results in water acidification research. However, in the same experiment nephrocalcinosis was only observed at CO₂ levels above 8 mm Hg (Fig. 10.9).

10.5.4 Gas supersaturation

Because of the high solubility of CO₂, increases in atmospheric level of the gas will have a negligible impact on gas supersaturation or GBD. This is not the case for climate change heating. For waters initially saturated, RCP8.5, 50 percentile temperature increases, the resulting Δ Ps are in the range of 40–67 mm Hg at year 2100. Estimated reductions in DO could reduce this Δ P by 10–20 mm Hg. For sensitive species, climate change impacts on gas supersaturation may occur in the 2060–2090 time period. More serious impacts could be expected for the 83 or 95 percentile temperature increase for RCP8.5. The potential impacts of climate change

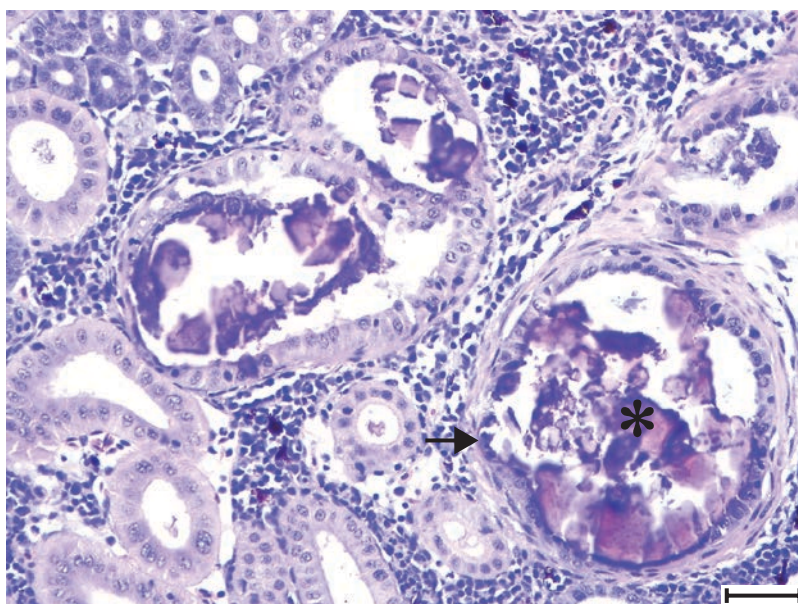


Fig. 10.9. Histopathology of nephrocalcinosis in Atlantic salmon showing calcium-containing deposits in the excretory tissues of the kidney (*) and necrosis of tubule epithelium (→). Section stained with haematoxylin and eosin. Bar, 50 μ m. (Photograph taken by A.B. Olsen.)

on gas supersaturation may be more serious in aquaculture because of limited water depth for hydrostatic compensation. Species sensitive to gas supersaturation include small freshwater and seawater fish larvae and species subject to swim-bladder or ocular damage from gas concentrating mechanisms. More care may be needed in the control of total gas pressures for these systems.

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11

The Immune System: Effects of Water Temperature and Acidification

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11.1 Introduction

The piscine immune system is similar to that in mammals and birds. It is organized at two levels: (i) an innate immune component; and (ii) an adaptive immune defence component. Protection via innate immunity depends upon recognition of common molecular structures of invading organisms. Moreover, it can act rapidly (within minutes–hours). The acquired component takes longer to react and it is characterized by specific antigen recognition and memory development. Specific responses usually require between weeks and months to build up adequate protection against pathogens. Specific receptors such as immunoglobulin (Ig) as well as other members of the ‘Ig superfamily’ occur in fish while classic Ig molecules have not been detected in invertebrates. This chapter provides a general overview of the defence mechanisms in fish, its interactions with the neuroendocrine system and the influence of environmental factors such as water temperature and acidification on its function. For general literature on innate and adaptive immunity in fish please see [Tables 11.1](#) and [11.2](#).

11.2 Innate and Adaptive Immunity

11.2.1 Epithelial barriers

The first line of defence includes structures (e.g. epithelial surfaces of skin, gills, gut) which form stable physical and/or chemical barriers against invading microorganisms. It is of prime importance for a fish to maintain the integrity of its covering epithelia because they are important in defence and for osmoregulation. Hence wound healing is a remarkably rapid process in fish. Normal epithelia

are covered by a mucus layer secreted by goblet cells. The most important function of mucus is to prevent the attachment of viruses, bacteria, fungi and parasites to epithelial surfaces. Moreover, mucus contains antimicrobial peptides (AMP) (Roussel and Delmotte, 2004). It is important to mention that AMP are also present in other tissues and organs and play a crucial role in the first line of defence against invading pathogens in fish (Masso-Silva and Diamond, 2014; Katzenbach, 2015). Numerous AMP have been discovered in fish and they include pleurocidins (Douglas *et al.*, 2001), heptacidins (Douglas *et al.*, 2003) and piscidins (Silphaduang *et al.*, 2006; Fernandes *et al.*, 2010).

11.2.2 Lectins

Lectins (or natural precipitins or agglutinins) in fish are cross-linking carbohydrate moieties on the surface of xenogeneic erythrocytes or bacteria. They are probably important in neutralizing bacterial secretions (e.g. exotoxins) or in immobilizing microorganisms and hence will facilitate phagocytosis (Fletcher, 1982). Fish lectins are not structurally related to Ig, but resemble plant or invertebrate agglutinins.

11.2.3 Lysozyme

This enzyme is found in fish mucus, serum and eggs (Ellis, 1999) and is able to digest the peptidoglycan layer of bacterial cell walls. Lysozyme is produced by macrophages and neutrophilic granulocytes (Murray and Fletcher, 1976) and is bactericidal for pathogens such as *Aeromonas salmonicida* and *Aeromonas hydrophila* (Ellis, 1999).

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Table 11.1. Reviews on innate immunity in teleost fish.

Subject	References
General reviews on innate immunity	Fletcher (1982), Yano (1996), Ellis (2001), Magnadóttir (2006), Aoki <i>et al.</i> (2008), Rebl and Goldammer (2018)
Cells and tissues	Ellis (1977)
Phagocytes	
Monocytes, macrophages	Secombes and Fletcher (1992)
Granulocytes	Ainsworth (1992)
B cells	Sunyer (2012)
Thrombocytes	Nagasawa <i>et al.</i> (2014)
Non-specific cytotoxic cells	Evans <i>et al.</i> (2001)
Natural killer (NK) cells	Fischer <i>et al.</i> (2013)
Molecules	
Antimicrobial peptides (defensins, hepcidins, piscidins, mucins, pleurocidins)	Roussel and Delmotte (2004), Silphaduang <i>et al.</i> (2006), Masso-Silva and Diamond (2014), Katzenback (2015)
Lectins (natural agglutinins)	Fletcher (1982)
Lysozyme	Murray and Fletcher (1976)
Cytokines	Zou and Secombes (2016)
Interferon	De Kinkelin <i>et al.</i> (1982)
Complement	Nakao <i>et al.</i> (2011)

Table 11.2. Reviews on adaptive immunity in teleost fish.

Subject	References
General reviews on adaptive immunity	Woo (1992), Manning (1994), Manning and Nakanishi (1996), Kaattari and Piganelli (1996), Van Muiswinkel and Vervoorn-Van Der Wal (2006), Rombout <i>et al.</i> (2011), Zhu <i>et al.</i> (2013), Van Muiswinkel and Nakao (2014), Castro and Tafalla (2015), Dixon <i>et al.</i> (2016)
Cells, tissues and organs	Ellis (1977), Zapata <i>et al.</i> (1996), Rombout <i>et al.</i> (2005)
B cells	Kaattari (1992)
T cells	Nakanishi <i>et al.</i> (2015)
Cytotoxic cells	Nakanishi <i>et al.</i> (2011)
Phagocytes	Secombes and Fletcher (1992)
Cell cooperation	Ye <i>et al.</i> (2013)
Molecules	
Immunoglobulins	Du Pasquier (1982), Wilson and Warr (1992), Pilström <i>et al.</i> (1998), Sunyer (2013), Ye <i>et al.</i> (2013)
Cytokines	Secombes (1991), Laing and Secombes (2004), Alejo and Tafalla (2011), Zou and Secombes (2016)

11.2.4 Interferon

Interferon (IFN) is a cytokine which is produced by many cell types in response to viral infections. It increases the resistance of host cells to different viruses by inducing the expression of proteins which inhibit the translation of viral mRNA. IFN in teleosts is species specific, for example IFN produced by rainbow trout (*Oncorhynchus mykiss*) does not protect cyprinid cells under *in vitro* conditions. *In vivo* synthesis of IFN during a viral infection peaks after

2–3 days and usually precedes the virus neutralizing effects of circulating antibodies, which appear 1 or 2 weeks later (De Kinkelin *et al.*, 1982). It is interesting that type I and type II IFN can be distinguished in rainbow trout based upon acid stability (pH 2) and relative temperature resistance (60°C) (Secombes, 1991). IFN activity has been demonstrated in a number of fish species, for example rainbow trout, Atlantic salmon (*Salmo salar*) and halibut (*Hippoglossus hippoglossus*) (Robertsen, 1999).

11.2.5 Complement

There are a number of excellent studies describing that most classes of fish possess a lytic complement system (Nakao *et al.*, 2011). Several groups of workers have shown that C1–C9 are present in carp (*Cyprinus carpio*) plasma (Yano, 1996; Nakao and Yano, 1998) and C5 in rainbow trout (Nonaka *et al.*, 1981). Important comparative studies have also been done by Sunyer and Lambris (1998) and Holland and Lambris (2002) in the USA. They mentioned that, unlike homoiotherms, several fish species possess multiple forms of complement components (C3 and factor B) that are structurally and functionally more diverse than those of higher vertebrates. Furthermore, it is noteworthy to mention that the alternative pathway is the protective mechanism against hemoflagellate parasites (*Cryptobia*) in naive fish (Woo, 1992). The classical pathway turned out to be important in acquired immunity after survival of parasitic infections or against bacteria, such as *Vibrio anguillarum* (Boesen *et al.*, 1999).

It is tempting to speculate the complement pathway predominates in fish. Yano suggested that the alternative pathway in fish is more active than in mammals (Yano, 1996). However, this may also depend on the ambient temperature (season), age or condition of the animals (Ellis, 2001; Magnadóttir, 2006). There is also evidence for a lectin complement pathway in fish (Fujita, 2002). These studies, taken together, suggest that all mammalian complement factors (C1–C9, B, D) are present in teleost blood and that all known pathways operate in fish (Nakao *et al.*, 2011).

11.2.6 Phagocytic cells

Macrophages and neutrophilic granulocytes are the principal phagocytic cells in fish (Secombes and Fletcher, 1992; Verburg-Van Kemenade *et al.*, 1994). Upon stimulation these cells phagocytose antigenic material and/or exert cytotoxic activity. The killing of intracellular or extracellular pathogens is based upon the release of a number of reactive oxygen species and nitric oxide (NO) (Campos-Perez *et al.*, 2000). It has been shown that macrophages play a key role in the defence against blood parasites in cyprinid fish (Joerink *et al.*, 2004). In one of these experiments carp were infected with *Trypanoplasma borreli* or *Trypanosoma carassii* and the activation state of the head kidney leucocytes was determined.

The results showed that *T. borreli*-infected carp were more prone to increase nitrite (marker for NO release) by classically activated macrophages while *T. carassii*-infected fish were more prone to increase arginase activity by alternatively activated macrophages (Joerink *et al.*, 2006). This is a clear indication for macrophage polarization in immune responses against parasites.

Phagocytosis of antigenic material by macrophages is not only an activity of the non-specific innate defence system but can also be an initial step in the specific adaptive immune response. As in mammals, we are probably dealing with subpopulations of mononuclear phagocytes, which differ in function. In this respect it is interesting to mention that macrophages from immune fish are more active in phagocytosis than those from control animals. This is probably due to opsonization of antigens by antibodies or due to metabolic activation of the macrophages (Griffin, 1983). Elegant studies by Rombout and Verburg-Van Kemenade and their teams (Rombout and Van den Berg, 1989; Koumans-Van Diepen *et al.*, 1994) have shown that most macrophages from the hindgut of carp are binding purified Ig, which is an indication for Fc receptors on the surface of these cells. This is an example of cooperation between the innate immune system (phagocytes) and the acquired immune system (Ig molecules). A new paradigm on phagocytes emerged by the finding of phagocytic B cells in teleosts, suggesting that phagocytosis is a leucocyte function with ancient origin, and B cell phagocytosis plays a significant protective role especially in lower vertebrates like teleosts (Sunyer, 2012). There are also indications that fish thrombocytes are active in phagocytosis (Nagasawa *et al.*, 2014).

11.2.7 Non-specific cytotoxic cells

Studies in channel catfish (*Ictalurus punctatus*) reveal the presence of non-specific cytotoxic cells (NCC) in these bony fish (Graves *et al.*, 1984; Evans and Jaso-Friedmann, 1992; Evans *et al.*, 2001). The monocyte-like NCC show a clear *in vitro* lytic activity against certain transformed mammalian cell lines. NCC have been shown in the blood, spleen and head kidney of several teleosts. NCC are probably involved in killing protozoan- and virus-infected cells. In addition to NCC distinct natural killer (NK) cells have been isolated and cloned from catfish blood (Shen *et al.*, 2004). These NK cells kill allogeneic targets, but are negative for markers defining neutrophils,

monocytes and NCC. For a thorough review on NK cells please see Fischer *et al.* (2013).

11.2.8 Lymphoid cells and organs

Lymphocytes are essential to the acquired immune response because they express the Ig and T cell receptor (TCR) molecules as antigen-specific-recognition units. Lymphocyte heterogeneity (T cells and B cells) in fish has been demonstrated in hapten-carrier studies (Stolen and Mäkelä, 1975), by using monoclonal antibodies (Secombes *et al.*, 1983) and by functional tests for cell cooperation (Miller *et al.*, 1987).

The thymus, head kidney (pronephros), trunk kidney (mesonephros), spleen and intestine contain high numbers of leucocytes (Rombout and Van den Berg, 1989; Rombout *et al.*, 1989; Fig. 11.1). Considerable numbers of leucocytes are also found in skin and gills (Iger and Wendelaar Bonga, 1994), which indicates that a mucosal immune system is well developed in fish (Rombout *et al.*, 2011, 2014). Bone marrow, bursa of Fabricius, Peyer's patches and lymph nodes, which are present in birds and/or mammals have not been found in fish. The spleen of bony fish is an erythropoietic and a secondary lymphoid organ (Van Muiswinkel *et al.*, 1991), whereas the thymus is a primary lymphoid organ mainly involved in T cell differentiation (Zapata *et al.*, 1996). The kidney (pronephros and mesonephros) is probably analogous to mammalian bone marrow (Lamers, 1985; Zapata *et al.*, 1996). Therefore, it may function as a primary organ (blood cell formation, B-lymphocyte differentiation) but also as a secondary organ (memory cell and plasma cell development).

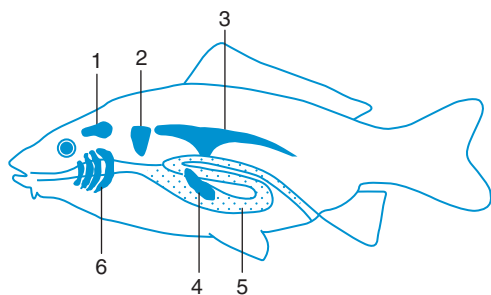


Fig. 11.1. The main lymphoid organs in teleost fish. 1 = thymus; 2 = head kidney (pronephros); 3 = trunk kidney (mesonephros); 4 = spleen; 5 = intestine; 6 = gills and skin. (Modified after Lamers, 1985, with permission.)

11.2.9 Ig structure

The major Ig in bony fish consists of heavy (H) chains and light (L) chains and hence is similar to that in other vertebrates. The native Ig molecule (Fig. 11.2) of fish is usually a tetramer with four structural units (H₂L₂)₄. It contains $4 \times 2 = 8$ antigen binding sites and has a molecular weight between 600 kDa and 900 kDa (Pilström *et al.*, 1998). The molecule is usually called immunoglobulin M (IgM) because of its high molecular weight and polymeric structure. However, the mammalian IgM is a pentamer with five structural units (H₂L₂)₅. The amino acid sequence of the four constant domains in the H chain (C_H) shows only 24% homology with the mouse μ chain (Ghaffari and Lobb, 1991). Interesting enough, the V_H genes of rainbow trout (Matsunaga *et al.*, 1990) show much higher amino acid sequence identity (45–60%) with mammals than the C domain genes. In other words the antigen-binding Fab part of the Ig molecule is probably better conserved in evolution than the ‘so-called’ constant part of the same molecule.

11.2.10 Ig isotypes

Although low molecular weight Ig has been reported in teleosts (Clem and Mclean, 1975; Lobb and Clem, 1981a), any structural or functional equivalency to mammalian IgG has not been found (Wilson and Warr, 1992). Moreover, the existence of a separate mucosal Ig (sub)class in bile and mucus of sheephead (*Archosargus probatocephalus*) (Lobb and Clem, 1981b) and carp (Rombout *et al.*, 1993) has been described. It was later confirmed that fish possess a separate mucosal Ig class, called IgT (Zhang *et al.*, 2010; Sunyer, 2013). Two related subclasses of specific antibodies, called IgZ, were described by Ryo *et al.* (2010). These subclasses show a functional difference in their response to blood and mucosal parasites in fish. Moreover, another chimeric Ig heavy chain sharing similarities with IgD has been found in channel catfish (Wilson *et al.*, 1997) and Atlantic salmon (Hordvik *et al.*, 1999).

11.2.11 Cell cooperation and cytokines

The acquired immune response in fish shows the expected characteristics of specificity and memory. At the start of the humoral (Ig) response it takes some time before the first specific antibodies

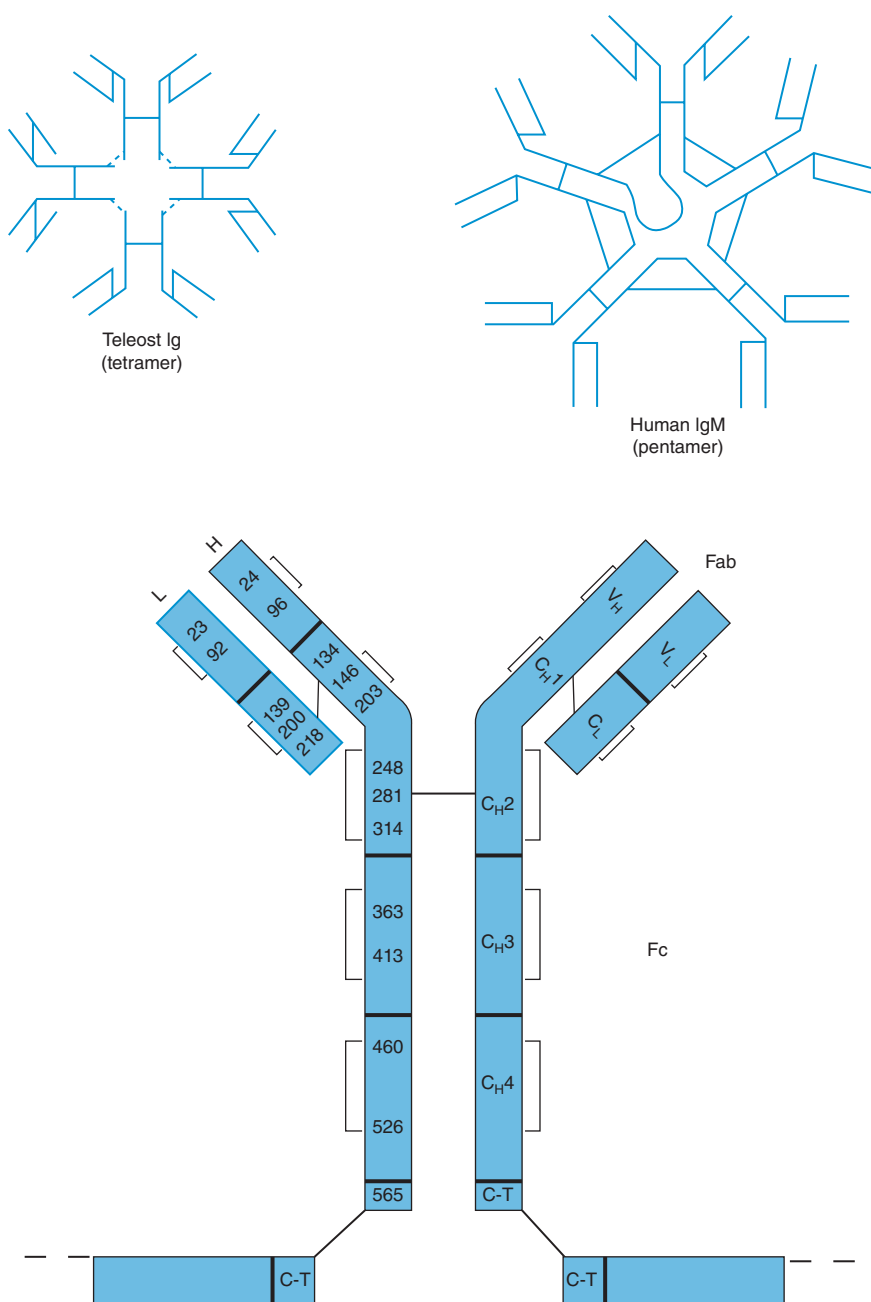


Fig. 11.2. Schematic representation of secreted teleost and human immunoglobulin M (IgM). The teleost immunoglobulin (Ig) molecule (upper left) is composed of equimolar amounts of heavy (H) and light (L) chains. These are assembled to produce a tetrameric molecule, as opposed to the pentameric human IgM (upper right). Each monomer (lower centre) possesses five domain heavy chains ($V_H + C_H$ 1–4) and two domain light chains ($V_L + C_L$). The brackets and lines depict potential intradomain and interchain disulfides, respectively. Numerals refer to positions of cysteine residues. C-T, C-terminal tail piece; Fab, fraction antigen-binding; Fc, fraction crystallizable. (From Kaattari and Piganelli, 1996, with permission.)

appear in the circulation. This lag phase is needed for antigen processing and cell cooperation between distinct leucocyte populations (accessory cells, B and T cells). Accessory cells (monocytes and macrophages) process different antigens and present the processed antigenic determinants in association with major histocompatibility complex (MHC) class II molecules to lymphocytes. We know from mammalian studies that the TCR on the membrane of a T helper (Th) cell is important for the recognition of the antigenic determinant. It took a long time before the presence of the TCR on lymphoid cells of fish could be proven. The group of Charlemagne was the first to provide evidence for TCR genes in rainbow trout (Partula *et al.*, 1996). During the interaction between macrophages and Th cells other molecules such as CD3 and CD4 are also essential as coreceptors. Activated macrophages will secrete interleukin(IL)-1, which is essential for the induction of the response by activating Th cells. Th cells regulate the proliferation and differentiation of B cells into Ig-secreting plasma cells by producing IL-2 and other interleukins. Most of these B, Th and accessory cell functions have been verified by using monoclonal antibodies and functional *in vitro* tests for channel catfish (Miller *et al.*, 1985, 1987) or for carp (Caspi and Avtalion, 1984).

Surprisingly, the apparently old and conserved IL-system exhibits a low degree of homology among vertebrate species when its ligands are compared at the level of amino acid sequences (approximately 30% homology between the human and teleost forms of IL-1 β and tumour necrosis factor alpha (TNF- α)). On the other hand, the secondary and tertiary structure of the IL-1 molecule appears quite conserved: Secombes and co-workers have shown that the trout IL-1 sequence can be superimposed on the human crystal structure for IL-1 β (Secombes *et al.*, 1998). It would appear that in an evolutionary context the conservation of three-dimensional structure is more important for cytokine function than its primary sequence. In recent years a variety of cytokine sequences was elucidated for several fish species. Fibroblast growth factor (FGF) and some other chemokines have been cloned from a number of fish species (Secombes *et al.*, 1999; Alejo and Tafalla, 2011). Several isoforms of the anti-inflammatory cytokine TGF- β have been described for fish and of the pro-inflammatory cytokines IL-1 β and TNF- β sequences are known (Secombes *et al.*, 1999). Zou *et al.*

(1999) published the first teleost sequence for rainbow trout IL-1 β in 1999. This was followed in 2000 by the IL-1 β sequence for common carp (Fujiki *et al.*, 2000). A second IL-1 β sequence was found for rainbow trout (Pleguezuelos *et al.*, 2000) and for carp in the following year (Engelsma *et al.*, 2001). An explanation for the existence of two related but distinct forms may be the tetraploidization event that occurred (independently) in some fish species during evolution. Elegant three-dimensional models of IL-1 β and IL-1 receptor type I from trout and sea bass (*Dicentrarchus labrax*) were predicted by comparison with those available from humans or mice (Scapigliati *et al.*, 2004). The multiple forms of IL-1 β and the presence of at least two types of receptors indicate that the complexity of the IL-1 system in teleosts is similar to that in mammals. Functional aspects of TNF- α action in fish were demonstrated using human recombinant TNF- α in rainbow trout macrophage (Knight *et al.*, 1998) and assaying for hepatocyte serum amyloid A expression (Jørgensen *et al.*, 2000). TNF- α sequences have been published for Japanese flounder (*Paralichthys olivaceus*) (Hirono *et al.*, 2000). While most teleost cytokine sequences are available, functional information on cytokines in neuroendocrine communication in teleosts is still limited. IL-1 β is the best-studied teleost cytokine with considerable importance and potency in the communication between the neuroendocrine system and the immune system (see Weys *et al.*, 1999; Verburg-Van Kemenade *et al.*, 2017).

11.2.12 Major histocompatibility complex (MHC)

During the last 30 years an impressive amount of information has become available on class I and class II loci in bony fish. Using polymerase chain reaction (PCR) Kurosawa and colleagues were able to demonstrate that the genome of carp contains nucleotide sequences showing considerable homology with MHC class I and II sequences in man and mice (Hashimoto *et al.*, 1990). Subsequently, classical class I, class II and/or β 2-microglobulin genes have been found in carp (Stet *et al.*, 1993), rainbow trout (Hansen *et al.*, 1999), medaka (*Oryzias latipes*) (Naruse *et al.*, 2000) and Atlantic salmon (Grimholt *et al.*, 2002). An important observation in all the bony fish studied is they are different from that in mammals – the class I loci are not linked to class II loci, but are on different linkage

groups. In other words, an MHC does not exist as a gene complex in fish. Several suggestions have been made to explain the absence of linkage between these class I and class II genes (Kuroda *et al.*, 2002). For example, duplication of parts of a chromosome bearing the MHC could have taken place followed by translocation and subsequent loss of certain loci or class II loci were translocated from a prototype MHC to other chromosomes in the ancestor of fish. It has been suggested that unlinked MHC class I and II genes could have an evolutionary advantage (Stet *et al.*, 2003). The offspring in mammals are endowed with only four possible MHC genotypes (haplotypes) per pair. Local populations usually show only a small number of MHC haplotypes. This limited diversity could provide a risk when environmental circumstances change or new diseases arise. This risk could only be counteracted by investing a relatively large amount of resources (care, energy) in a relatively small number of offspring. However, fish usually have high numbers of offspring – up to thousands or even millions. Mortality in fish can be over 80% in early life, which can be due to predation, diseases or environmental (climatic) factors. Fish with unlinked MHC genes have the ability to endow their offspring with high numbers of genotypes, which will increase the chance that at least some individuals will survive.

11.2.13 Humoral immunity

The kinetics of the humoral response in bony fish has been studied in detail (Rijkers, 1982; Kaattari and Piganelli, 1996). The length of the lag phase, exponential phase and decay phase following immunization may be influenced by environmental factors (e.g. temperature, stress), type of antigen, antigen dose, route of application, age and fish species (see Section 11.3). Injection of an optimal dose of sheep red blood cells (SRBC) into carp (at 24°C) evokes peak numbers of antibody-forming cells in spleen and kidney after 9–10 days (Rijkers *et al.*, 1980), but rainbow trout (at 12–17°C) need 14–15 days for the same response (Chiller *et al.*, 1969). Studies in European eel (*Anguilla anguilla*) (Esteve-Gassent *et al.*, 2003) kept at 26°C have shown that the antibody response to *Vibrio vulnificus* in mucus is faster (peak at 3–4 days) than in serum (peak at 7 days or later). After injection of SRBC in carp at 20°C the first antibody-producing plasma cells appear in the spleen and kidney

around the first week after immunization followed by a peak in the second week. Circulating antibody titres peak later due to the relatively long half-life of the Ig molecule (Harrell *et al.*, 1975). After a second contact with the same antigen, the lag phase is shorter and the response is accelerated. Moreover, higher numbers of plasma cells or titres are reached. However, an Ig isotype switch is not observed and the increase in antibody affinity is limited when compared with mammals (Arkoosh and Kaattari, 1991; Kaattari, 1992).

11.2.14 Cellular immunity

Cellular immunity in fish has been studied *in vitro* using mixed leucocyte reactions (MLR), cytokine production, stimulation of DNA synthesis by T cell mitogens or antigens (Kaastrup *et al.*, 1988; Secombes, 1991). *In vivo* studies include delayed type hypersensitivity reactions or graft rejection (Rijkers, 1980; Manning and Nakanishi, 1996). The *in vivo* kinetics of specific cellular responses have been extensively studied by following the fate of transplanted scales or skin (Borysenko and Hildemann, 1970; Rijkers and Van Muiswinkel, 1977). The cellular reactions, which occur at the grafting site, are essentially the same as in mammals. The graft-invading host cells are lymphocytes and macrophages. Second-set grafts are rejected more rapidly than first-set grafts. Specific cytotoxicity has also been shown in *in vitro* approaches by using modified autologous cells as targets (Verlhac *et al.*, 1990). It was demonstrated that the response of primed leucocytes to autologous trinitrophenyl (TNP)-modified target cells was considerably greater than against allogeneic TNP-modified cells suggesting that MHC restriction was involved. In mammals, cytotoxic T cells recognize antigen in association with self (MHC class I) surface molecules.

11.3 Environmental Effects

11.3.1 Temperature

In cold-blooded animals such as fish, the metabolic activity is directly influenced by the ambient water temperature. The effects of temperature on antibody synthesis have been known for a long time (Nybelin, 1935; Cushing, 1942; Bisset, 1947; Ambrosius and Schäker, 1964). From studies in eels and cyprinids the general conclusion is that a

water temperature between 16°C and 28°C is required for optimal synthesis of agglutinating antibody, whereas no or very delayed antibody production occurs below 15°C. However, O'Neill (1980) showed that brown trout (*Salmo trutta*) were able to develop a specific and high antibody response between 9°C and 15°C. In other words: the optimal temperature range for immune responses can be different when comparing fish families. Rijkers *et al.* (1980, 1981) studied the kinetics of the antibody-forming cell response in carp adapted to different temperatures between 12°C and 28°C. They showed that lowering temperatures caused a delay of the peak in the primary response. The anamnestic character of the secondary response was clearly demonstrated at 24°C and 20°C, but less clear or absent at 18°C or lower. The relationship between temperature and the humoral response in carp is shown in Fig. 11.3 (Rijkers *et al.*, 1980). This relationship matches the effect of temperature on allograft survival in goldfish (*Carassius auratus*) (Hildemann and Cooper, 1963). Avtalion and Clem (1981) studied the effects of temperature on the antibody production in common carp and Nile tilapia (*Oreochromis niloticus*) against bovine serum albumin and hapten-carriers. They showed that synthesis and release of antibody could take place at low temperatures ($\leq 12^\circ\text{C}$) if fish were kept at high temperatures (25°C) during the early phase of the response. It was suggested that antigen processing and subsequent cooperation between macrophages, Th and B cells is a temperature-sensitive event which lasts 3–4 days in these warm-water fish. The temperature sensitivity of T cells was confirmed by Miller and Clem (1984) who showed that low temperatures inhibited the generation of putative carrier-specific memory Th cells from virgin Th cells. Cytokine production in rainbow trout is also inhibited at non-permissive temperatures. Again it is the T cell that is the temperature-sensitive cell, the macrophage function itself remaining intact at low temperatures when stimulated with macrophage activating factor (MAF) (Hardie *et al.*, 1994). It is possible that innate immunity may compensate for the loss of acquired immunity at lower temperatures. For example, low temperatures inhibiting the mitogenic effect of phytohaemagglutinin (PHA) on carp T cells were found to enhance NCC activity (Le Morvan-Rocher *et al.*, 1995). Nikoskelainen *et al.* (2004) showed that innate immunity (respiratory burst

activity, lytic activity of total and alternative complement pathways) in rainbow trout was still working, but responding at a lower level in animals acclimatized to lower temperatures (5–10°C). The normal function of fish lymphocytes at different temperatures is highly dependent on homoviscous adaptation of membrane lipids (Abruzzini *et al.*, 1982). It is likely that the fatty acid composition (unsaturated versus saturated) determines the fluidity and permeability of membranes as well as the activity of membrane-associated receptors and enzymes. Sheldon and Blazer (1991) working with channel catfish at optimal (28°C) and suboptimal (19°C) temperatures observed a positive correlation between the bactericidal activity of macrophages and the level of highly unsaturated fatty acids in the diet. This opens new perspectives for the improvement of disease resistance of fish at lower temperatures. Additional information on the interaction between temperature and the immune system of fish are in excellent reviews (see Anderson, 1996; Le Morvan *et al.*, 1998; Golovanov and Mikryakov, 2011; Golovanov, 2015; Abram *et al.*, 2017).

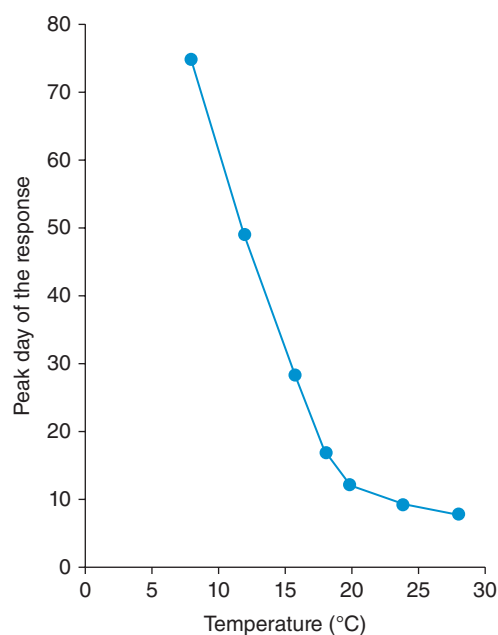


Fig. 11.3. The relationship between the water temperature and the speed (peak day) of the antibody response in common carp. (From Rijkers *et al.*, 1980, with permission.)

11.3.2 Acclimatization

Temperature is one of the most important environmental factors which determine development, reproduction, growth, behaviour and health of fish. In most cases fish families prefer a certain temperature range (e.g. 10–17°C for Cottidae, 11–16°C for Salmonidae, 16–18°C for Gasterosteidae, 20–28°C for Cyprinidae and 28–32°C for Cichlidae) (Golovanov, 2006). Within the species-specific temperature range juvenile fish usually prefer a slightly higher temperature (2–4°C) than adult fish (Golovanov, 2013). In most cases seasonal or climate changes will require that the animals adapt to the new condition even if the ambient temperature is much lower or higher than the preferred temperature. In this acclimatization process fish are forced to follow the changes in the environmental conditions passively. During this process there are changes in factors influencing enzyme structure and activity, membrane composition and gene expression. In addition, changes in mitochondrial densities have been reported for the liver, brain and gill tissue during acclimatization (Johnston and Dunn, 1987). The duration of the whole process can be in the order of several weeks, even if the change in temperature is only a few degrees Celsius (Golovanov, 2006).

A special form of thermal regulation is the behavioural fever response. In this particular case an acute change in the preferred temperature occurs when a pathogen is recognized followed by an immune response (Kluger, 1979; Reynolds and Casterlin, 1979; Golovanov, 2013). Provided that the possibility is available, the infected fish will seek water temperatures a few degrees higher and stay there for 1 day or more. In goldfish the survival after infection with *A. hydrophila* was the highest in the group which was allowed to do this (Covert and Reynolds, 1977). Comparable results were obtained in Nile tilapia challenged with another bacterium, *Streptococcus iniae* (Cerqueira *et al.*, 2016). In recent work on behavioural fever in zebrafish (*Danio rerio*) a viral infection was simulated by injecting a synthetic double-stranded RNA. This ‘viral’ challenge resulted in a 3°C upward shift in thermal preference during just 1 day. In these animals an upregulation of antiviral genes was observed using transcriptome analysis of whole brain (Boltaña *et al.*, 2018). In other experiments in common carp infected with cyprinid herpesvirus 3 (CyHV-3) the control fish maintained at 24°C all

died, whereas those kept in tanks encompassing a 24–32°C gradient moved to the highest temperature and survived (Rakus *et al.*, 2017). An additional observation was that CyHV-3 is expressing a soluble decoy receptor for TNF- α . This receptor bound the TNF- α produced by the infected fish, which had a negative effect on behavioural fever and allowed the virus to multiply.

11.3.3 Acidification

We know that climate change is primarily caused by increased levels of atmospheric CO₂. Both warming and acidification are – at least to a great extent – the result of high CO₂ levels (Bresolin de Souza *et al.*, 2014; Stiasny *et al.*, 2016). The effect of temperature on the immune system of fish has been studied in detail for a long time (see Sections 11.3.1 and 11.3.2). However, information on the effect of acidification (or a combination of acidification and temperature) is limited.

Holopainen and Oikari (1992) observed ionoregulatory imbalance, chronic stress (elevated cortisol level) and high mortality of crucian carp (*Carassius carassius*) in a pond, where the pH decreased from 6.0 to 4.0 during 4 months. Normal cortisol values and good survival were observed in another pond, where the pH 6.0 stayed constant during the same period. Iger *et al.* (1994) studied the changes in cell populations of the skin and plasma cortisol levels after exposure of rainbow trout to water of pH 5. After 1 day in acid water a significant elevation of the cortisol level was observed, but by day 7 all was normal again. Around day 7 many leucocytes (mainly macrophages and lymphocytes) were seen in the epidermis and high numbers of these lymphocytes underwent apoptosis. Exposure of juvenile common carp to water of pH 4.5 induced a rapid (within 1 day) peak in plasma cortisol and a significant decrease in IgM levels after 7 days (Ikuta *et al.*, 2000). Comparable observations were published by Nagae *et al.* (2001). They studied the effect of chronic low pH exposure (pH 4.5) on endocrine and immune functions in the common carp. Plasma cortisol levels increased 3 h after acidic exposure and stayed higher than those in the controls until the end of the experiment at 4 weeks. Plasma IgM levels were significantly lower than normal at 1 week from the start, but were back to normal after 4 weeks of acidic exposure. Phagocytosis by blood leucocytes decreased significantly at 1 week of exposure and stayed low until the end at

4 weeks. In another study proteomic analysis of gills and blood plasma of halibut showed that high CO₂ treatment induced the upregulation of immune system-related genes (e.g. complement component C3) and apoptosis signalling proteins (Bresolin de Souza *et al.*, 2014). It can be concluded that acidification can induce stress responses and disturbance of immune responses in fish.

11.3.4 Stress

It is obvious that human activities can affect fish welfare (e.g. in fisheries or aquaculture) but climate change can also have an effect. Tissue damage, physical exhaustion and immune suppression can occur as a result of these circumstances. It is possible that fish are exposed to stress even under natural circumstances due to poor water quality caused by unusual O₂, temperature and pH values. In fishes, as in mammals, the stress response comprises activation of the sympathetic nervous system as well as of the hypothalamus–pituitary–interrenal (HPI)-axis; the interrenal tissue in the head kidney of fish contains the equivalents (cortisol-producing cells and chromaffin cells) of the mammalian adrenals (Schreck, 1996; Wendelaar Bonga, 1997; Lange *et al.*, 2018). In response to hypothalamic release of corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) the pituitary enhances synthesis of proopiomelanocortin (POMC) and the release of its cleavage products. Adrenocorticotrophic hormone (ACTH) is a potent stimulator of cortisol production by the interrenal steroid-producing cells. Cortisol has both glucocorticoid and mineralocorticoid actions in fish (the type of response to cortisol is receptor dependent). As the head kidney combines glucocorticoid and catecholamine production with important immune features (e.g. lymphopoiesis and antibody production) the potential for paracrine modulation of immune responses by stress hormones is indicated. Effects of cortisol on the immune system of fish are generally similar to those in mammals (Weyts *et al.*, 1999; Harris and Bird, 2000; Yada and Tort, 2016; Verburg-Van Kemenade *et al.*, 2017). It should be mentioned that the level of resting cortisol and the ability of cortisol to suppress the immune system can differ depending on the life history stage of salmonid fish (Schreck, 1996; Fig. 11.4). This means that additional stress caused by temperature or acidification can have extreme immune suppressive effects in certain life phases. Other studies

suggest that chronic stress causes lymphocyte depletion in peripheral blood and lymphoid organs (Zapata *et al.*, 1992). Circulating lymphocyte populations decrease in number while neutrophilic granulocytes remain constant or increase (Ellsaesser and Clem, 1986; Ainsworth *et al.*, 1991; Morgan *et al.*, 1993). Lymphocyte proliferation is decreased after injection with cortisol (Espelid *et al.*, 1996) and *in vitro* antibody responses are impaired after cortisol administration (Carlson *et al.*, 1993). Reports on the effects of stress or cortisol on respiratory burst and phagocytosis are conflicting, but may reflect differences between fish species as well as differences in methodologies. Receptors for glucocorticoids have been demonstrated in salmon and carp leucocytes (Maule and Schreck, 1990; Weyts, 1998). In carp a differential effect of cortisol was demonstrated on lymphocytes and neutrophilic granulocytes under *in vitro* conditions. Activated B cells harvested from the blood are easily triggered to enter cortisol-induced apoptosis (Weyts, 1998). Moreover, compared with B cells from head kidney and spleen, circulating B cells are most affected by cortisol (Verburg-van Kemenade *et al.*, 1999). In contrast to the sensitivity of B cells to apoptosis signals, carp neutrophilic granulocytes are rescued from apoptosis by cortisol (Weyts, 1998), demonstrating dual actions of glucocorticoids in fish. A sudden drop in water temperature (9°C) can induce a stress response in carp (Engelsma *et al.*, 2003). The relative number of circulating B cells in the total leucocyte population dropped within 4 h after cold shock. In the head kidney an increase in the relative number of B cells was observed. However, granulocyte numbers showed an opposite reaction. The conclusion was that this was probably due to a redistribution of these cells over different organs. Moreover, the antibody response to a T cell independent antigen (TNP-LPS) was delayed after acute temperature stress. There are indications that not only mammalian but also fish leucocytes produce HPI-axis hormones. Ottaviani *et al.* (1998) demonstrated in goldfish the presence of immunoreactive CRH in the thymus. Channel catfish leucocytes (peripheral blood leucocytes; B and T cell lines) secrete ACTH (Arnold and Rice, 2000) both constitutive and CRH-driven. Thus, although research in this field is only starting, we anticipate that ‘stress hormones’ in fish are produced by leucocytes allowing bi-directional communication between the neuroendocrine system and the immune system (Fig. 11.5).

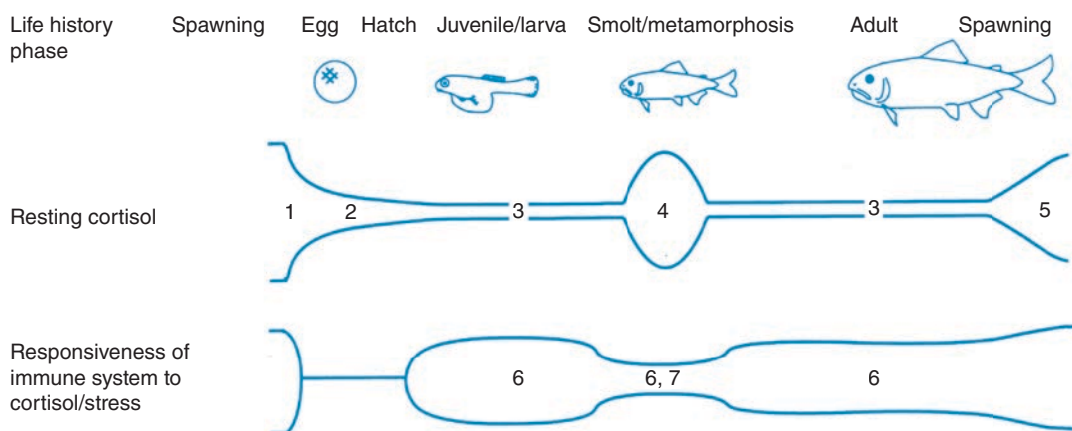


Fig. 11.4. Conceptualization of the pattern of resting cortisol levels and the ability of cortisol to suppress the immune system (greater height indicates more suppression) at different life history stages of salmonid fish. The height of each pattern reflects the magnitude of the concentration or of the response. This is shown on a relative scale and is not precise. Numbers refer to references that support the pattern at a particular stage: 1, Caldwell *et al.*, 1991; 2, Yeoh *et al.*, 1996; 3, Barton *et al.*, 1985; 4, Nagae *et al.*, 1994; Barton *et al.*, 1985; 5, Donaldson and Fagerlund, 1968; Maule *et al.*, 1996; 6, Maule and Schreck, 1990; Maule *et al.*, 1993; 7, Maule *et al.*, 1987, 1989; Muona and Sovio, 1992. (Modified after Schreck, 1996, with permission.)

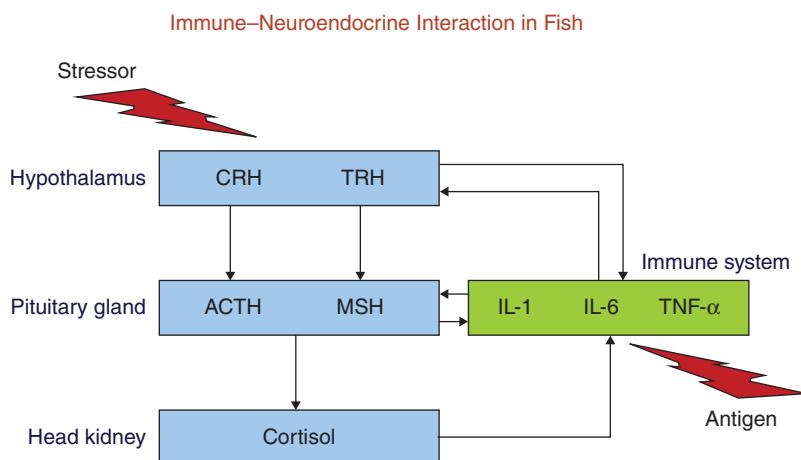


Fig. 11.5. Interaction between the stress response and the immune response in fish. During the stress response, neuropeptides including corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) control the release of pituitary hormones involved in the regulation of cortisol (ACTH, adrenocorticotrophic hormone; MSH, melanophore-stimulating hormone). The head kidney of fish contains equivalents (e.g. cortisol-producing interrenal cells) of the mammalian adrenal. High levels of cortisol may affect the expression of cytokine genes in cells of the immune system. Cytokines (e.g. interleukin (IL)-1, IL-6 and tumour necrosis factor alpha (TNF- α)) play an important role in the regulation of the immune response, but are also known to interact with the hypothalamus–pituitary–interrenal (HPI) axis. Administration of IL-1 in experimental fish can activate CRH neurons and stimulates the release of CRH illustrating immune–neuroendocrine interaction. (J. Metz, G. Flik and S.E. Wendelaar Bonga, Radboud University, Nijmegen, the Netherlands, 2005, personal communication).

11.4 Conclusions

During the last 30–40 years considerable progress has been made in describing and understanding the immune system of fish. Antigenic stimulation in fish evokes responses which are comparable to those in warm-blooded vertebrates. An effective innate immune system is present and acquired immune responses show the expected characteristics of specificity and memory. There are clear effects of environmental factors such as water temperature and/or acidification on fish immunity. Even small changes in temperature can evoke a stress response. Chronic stress has usually the most deleterious effect on immunity. It can be expected that the effects of climate change will be different depending on the biotope (ponds, rivers, ocean), adaptive capacity of a fish group/family, but also on the developmental stage of an individual fish.

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Climate Change and Non-infectious Fish Disorders

Edited by **Patrick T.K. Woo** and **George K. Iwama**

Freshwater, brackish and marine ecosystems are particularly impacted by the effects of climate change and global warming. A global rise in water temperature and acidification of the aquatic environment will continue even if we can significantly reduce the current output of greenhouse gasses. Increases in water temperature will affect the life cycle, physiology, behaviours, distribution and community structure of aquatic organisms, especially fish.

This important new text on climate change, and its effects on selected non-infectious disorders of fish, contains contributions by internationally recognized experts who have contributed significantly to our knowledge in this area. Comprehensive and thought provoking, the text details abiotic and biotic environmental changes associated with climate change and their effects on fish in tropical, subtropical and temperate waters. It proceeds to cover in detail developmental, physiological and metabolic disorders of fish.

Outlining both current and expected changes in aquaculture systems due to climate change, plus suggestions for further studies, this contemporary text is key reading for biologists, aquatic ecologists, fish health consultants, veterinarians, policy makers and all those involved in fish health and the environment.